



Microalgal biomass production and on-site bioremediation of carbon dioxide, nitrogen oxide and sulfur dioxide from flue gas using *Chlorella* sp. cultures

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ABSTRACT

The growth and on-site bioremediation potential of an isolated thermal- and CO₂-tolerant mutant strain, *Chlorella* sp. MTF-7, were investigated. The *Chlorella* sp. MTF-7 cultures were directly aerated with the flue gas generated from coke oven of a steel plant. The biomass concentration, growth rate and lipid content of *Chlorella* sp. MTF-7 cultured in an outdoor 50-L photobioreactor for 6 days was 2.87 g L⁻¹ (with an initial culture biomass concentration of 0.75 g L⁻¹), 0.52 g L⁻¹ d⁻¹ and 25.2%, respectively. By the operation with intermittent flue gas aeration in a double-set photobioreactor system, average efficiency of CO₂ removal from the flue gas could reach to 60%, and NO and SO₂ removal efficiency was maintained at approximately 70% and 50%, respectively. Our results demonstrate that flue gas from coke oven could be directly introduced into *Chlorella* sp. MTF-7 cultures to potentially produce algal biomass and efficiently capture CO₂, NO and SO₂ from flue gas.

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1. Introduction

Global warming, which is induced by an increase in the concentration of greenhouse gases in the atmosphere, is of great concern and has received increasing attention as natural sources of fossil fuels have become exhausted (Favre et al., 2009). Carbon dioxide (CO₂) is one of the main greenhouse gases (GHG) emitted into the atmosphere. Flue gases from power plants are responsible for more than 7% of world CO₂ emissions from energy use (Kadam, 2002), and steel plants are the single largest source of energy-related CO₂ emissions in the world (Gielen, 2003).

The efficient mitigation of GHG emissions is an international issue. Biological methods, particularly microalgal photosynthesis, have several merits, such as higher CO₂ fixation rates than terrestrial plants and no requirement for further disposal of the trapped CO₂. The incorporation of CO₂ into a biomass carbon source, such as carbohydrates and lipids, by microalgal fixation of CO₂ by photosynthesis is the most promising potential method for CO₂ sequestration from flue gas (Lee and Lee, 2003; Doucha et al., 2005; Wang et al., 2008; Brune et al., 2009; Yoo et al., 2010; Ho et al., 2011).

Microalgal biomass can be used for biofuel production by 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 (Brennan and Owende, 2010; Lee et al., 2010). Capturing CO₂ from industrial processes using microalgae and the subsequent utilization of the generated biomass for transportation needs would aid in achieving CO₂ sequestration and reducing our overall carbon emissions.

In general, the primary emission in flue gas is CO₂, which is present at concentrations ranging from 3% to 25% depending on the fuel source and the design of the plant (Packer, 2009). This CO₂ 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 CO₂ and the presence of inhibitory compounds such as NO_x and SO_x (Negoro et al., 1999; Lee et al., 2000). Temperature is also an inhibitory growth factor for outdoor microalgal cultivation. The temperature of the microalgal culture broth in the photobioreactors can increase to about 40 °C due to irradiation by sunlight. Feasibility of sequestering CO₂ from flue gas depends on either installing heat exchanger system or using thermophilic species (Kumar et al., 2011). Temperature control must be addressed if microalgae are used for large-scale outdoor cultivation (Béchet et al., 2010).

In the present study, an isolated thermal- and CO₂-tolerant mutant strain of *Chlorella* sp. was used in an on-site outdoor microalgal cultivation with flue gas aeration. The flue gas was generated

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from coke oven of a steel plant. The effects of flue gas aeration on the growth and lipid production of the *Chlorella* sp. mutant strain was investigated. Furthermore, on-site elimination efficiencies of CO₂, NO and SO₂ from the flue gas were evaluated.

2. Methods

2.1. Microalgal cultures, medium and chemicals

The microalga *Chlorella* sp. MTF-7 was originally obtained from the collection of Taiwan Fisheries Research Institute (Tung-Kang, Ping-Tung, Taiwan) and isolated in our laboratory by chemical mutagenesis. The *Chlorella* sp. MTF-7 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 MgSO₄ 7H₂O, 1.21 g L⁻¹ Tris-base, 1.83 g L⁻¹ CaCl₂ 2H₂O and 0.25 g L⁻¹ NaHCO₃, with 0.3% (v/v) macro elemental solution and 0.3% trace elemental solution. The macro elemental solution was 75 g L⁻¹ NaNO₃ and 5 g L⁻¹ NaH₂PO₄ H₂O. The trace elemental solution was 4.36 g L⁻¹ Na₂ EDTA, 3.16 g L⁻¹ FeCl₃ 6H₂O, 180 mg L⁻¹ MnCl₂ 4H₂O, 10 mg L⁻¹ CoCl₂ 6H₂O, 10 mg L⁻¹ CuSO₄ 5H₂O, 23 mg L⁻¹ ZnSO₄ 7H₂O, 6 mg L⁻¹ Na₂MoO₄ 2H₂O, 100 mg L⁻¹ vitamin B₁, 0.5 mg L⁻¹ vitamin B₁₂ and 0.5 mg L⁻¹ biotin.

2.2. Measurement of microalgal cells, growth rate

Biomass concentration (dry weight per liter) of cultures were measured according to the method reported previously (Chiu et al., 2009a). Regression equations of the relationship between optical density and cell dry weight were established and shown as follows:

$$y = 0.2529x - 0.0153, \quad R^2 = 0.9898$$

The value y is biomass concentration (g L⁻¹). This value was determined according the method previously reported (Chiu et al., 2009a). Microalgal cells were collected, centrifuged and washed with dideionized water. The washed microalgal pellet was dried at 105 °C for 16 h; afterward, the dried cells were for dry weight measurement. 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 if optical density was greater than 1.0.

The optical density was used to evaluate the biomass concentration of *Chlorella* sp. MTF-7 in each experiment. In the present study, we used biomass concentration (g L⁻¹) for the quantitation of *Chlorella* sp. MTF-7 cell density in the culture. The growth rate was measured and according the equation showed as follows:

$$\text{Growth rate} = \frac{W_f - W_i}{\Delta t}$$

where W_f and W_i is the final and initial biomass concentration, respectively. Δt is the cultivation time in days.

2.3. Experimental system of indoor photobioreactor

The microalgal cells were cultured in photobioreactors with a working volume of 800 mL (Chiu et al., 2008). The photobioreactors were placed in an incubator at 25 ± 1 °C with a surface light intensity of approximately 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 CO₂-enriched gas was premixed with air and pure CO₂ for the flue gas

experiments as a control gas. In the gas airstream, CO₂ concentration was adjusted to 2%, 10% and 25% for cultures as control experiments. The flue gas (approximately 25% CO₂, 4% O₂, 80 ppm NO and 90 ppm SO₂) was collected from coke oven in China Steel Corporation and was directly introduced into microalgal cultures. The gas flow rate was adjusted to 0.05 vvm (volume gas per volume broth per min) using a gas flow meter (Dwyer Instruments, Inc., Michigan city, IN, USA). The evaluation of tolerance to the flue gas in microalgal cultures, initial biomass concentration of *Chlorella* sp. MTF-7 cultures were approximately 0.2 g L⁻¹. The microalgal cells in each treatment were sampled every 24 h for determination of the biomass concentration.

2.4. Experimental system of outdoor photobioreactor

The outdoor photobioreactor was cylindrical and made of acrylic polymer. The column was 300 cm in length and 16 cm in diameter. The working volume of the photobioreactor was 50 L (Ong et al., 2010). The gas flow rate was adjustable using a gas flow meter. The source of flue gas was from a coke oven in China Steel Corporation (Kaohsiung, Taiwan). The concentrations of CO₂, O₂, NO and SO₂ in the flue gas were 23 ± 5%, 4.2 ± 0.5%, 78 ± 4 ppm and 87 ± 9 ppm (October 1–November 15, 2010), respectively. In the intermittent flue gas aeration, culture aeration was controlled by a gas switch, and a gas-switching cycle was performed with a flue gas inlet load for 30 min followed by air inlet load for 30 min (30 min flue gas/30 min air) during the day. The inlet and outlet loads were real-time monitored by a gas analyzer (AMETEK, Inc., Paoli, PA, USA) to determine the concentrations of CO₂, O₂, NO and SO₂.

2.5. Lipid extraction

Lipid extraction was according to the modified method previously reported (Chiu et al., 2009b). The microalgal cells were centrifuged and washed with deionized water twice, and obtained the dry biomass by lyophilization. The dried sample (200 mg) 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.

2.6. Microalgal lipid transesterification

The mixture of methanol (1.7 mL), sulfuric acid (0.3 mL) and chloroform (2.0 mL) was added to the microalgal oil, and heated at 90 °C for 40 min with thoroughly mixing during heating. The samples were then cooled to room temperature and mixed with 1 mL dideionized water. Finally, the organic (lower) phase containing fatty acid methyl esters (FAMES) was collected and the solvent was evaporated.

2.7. Fatty acid profile analysis

The fatty acid composition was determined FOCUS Gas Chromatograph (Thermo Fisher Scientific, Waltham, MA, USA) equipped with an flame ionization detector (FID) and trace GC capillary column (Thermo Fisher Scientific, Waltham, MA, USA), which was a cyanopropylphenyl based phase specifically designed for the separation of FAMES. A 30 m long column was used with a diameter of 0.32 mm and a 0.25 μm thick film. The amount of sample injected was 2 μL. The stripping gas was nitrogen 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 where it remained for 1 min, then rising from 150 to 180 °C at 10 °C min⁻¹, remaining at 180 °C for 3 min, then rising from 180 to 220 °C at 1.5 °C min⁻¹, remaining at 220 °C for 1 min, and finally rising from 220 to 260 °C at 30 °C min⁻¹, remaining at 260 °C for 5 min. 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, Waltham, MA, USA).

2.8. Chemical analyses

The inlet and outlet loads of airstreams were real-time monitored by a gas analyzer. The concentration of O₂, CO₂, NO and SO₂ in flue gas were measured using Landcom III portable gas analyzer (AMETEK, Inc., Paoli, PA, USA).

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, USA).

3. Results and discussion

3.1. Growth parameters of *Chlorella* sp. MTF-7 aerated with flue gas

3.1.1. Indoor culture experiments

In our previous study (Chiu et al., 2008), microalgal cell growth was significantly inhibited when wild-type microalgal *Chlorella* sp. cultures were aerated with gas containing a high concentration of CO₂ (>10% CO₂). Given the high concentration of CO₂ in flue gas (about 20–25% CO₂), the growth potential of the isolated microalga, *Chlorella* sp. MTF-7, when aerated directly with flue gas was first evaluated. In indoor culture experiments, batch cultures of *Chlorella* sp. MTF-7 were incubated for 6 days at 25 ± 1 °C under continuous cool white fluorescent light. The light intensity was approximately 300 μmol m⁻² s⁻¹ at the surface of the photobioreactor. The flue gas generated from coke oven of a steel plant was collected in a gas storage bag, and the gas was continuously introduced into the photobioreactor by an air blower.

Fig. 1 shows the growth curves of *Chlorella* sp. (wild-type, WT) and *Chlorella* sp. MTF-7 aerated with flue gas or CO₂-enriched gas (2%, 10%, or 25% CO₂ aeration) for 6 days. The growth potential of *Chlorella* sp. MTF-7 was significantly higher than that of *Chlorella*

sp. WT when aerated with flue gas or CO₂. The maximum biomass concentrations in *Chlorella* sp. MTF-7 cultures aerated with 2%, 10% or 25% CO₂ were 1.67, 1.50 and 1.32 g L⁻¹, respectively. The maximum biomass concentration was 2.40 g L⁻¹ in the batch cultures of *Chlorella* sp. MTF-7 aerated with flue gas. The average growth rates of the *Chlorella* sp. MTF-7 cultures aerated with flue gas or 2%, 10% or 25% CO₂ were 0.37, 0.25, 0.15 and 0.19 g L⁻¹ d⁻¹, respectively. The growth rates of the *Chlorella* sp. MTF-7 cultures aerated with flue gas were approximately 1.5-, 2.5- and 2.0-fold higher than those of the *Chlorella* sp. WT cultures aerated with 2%, 10% or 25% CO₂, respectively. These results indicated that *Chlorella* sp. MTF-7 could be cultured with flue gas aeration; the maximum biomass productivity was 0.64 g L⁻¹ d⁻¹ in the batch culture aerated with flue gas. The growth potential of *Chlorella* sp. MTF-7 cultures aerated with flue gas from the coke oven of a steel plant, which contained approximately 25% CO₂, 4% O₂, 80 ppm NO and 90 ppm SO₂, was higher than that of the cultures aerated with 2%, 10% or 25% CO₂-enriched gas without pH control. The high growth capacity of microalgae aerated with flue gas has been reported previously (Douskova et al., 2009). The volumetric concentration of CO₂ provided to the control culture was the same as the average concentration in the flue gas (11%). However, the growth rate of *Chlorella vulgaris* cultures aerated by flue gas from an incinerator was 48% higher than that of the control culture. The high concentration of CO₂ in flue gas was a major factor in microalgal growth (Yoo et al., 2010). In our previous study, a high initial density of *Chlorella* sp. could overcome the environmental stress induced by high CO₂ aeration and grow rapidly (Chiu et al., 2008). In this experiment, an initial high-density culture was used, and a gas-switching cycle operation was also introduced. In a high-density culture, the growth inhibition caused by the high CO₂ concentration in the flue gas is reduced, and the pH value of the culture can be stably maintained.

The NO_x present in flue gas inhibits microalgal growth (Lee et al., 2000). However, the toxic effect of NO can also be overcome by high-density cultures, and NO can be a nitrogen source for microalgal cultures. NO absorbed in the medium can be converted to NO₂ and then oxidized to NO₃, which can be utilized as a nitrogen source (Nagase et al., 2001). Gaseous NO can dissolve in the broth of microalgal cultures and can be taken up directly by algal cells through diffusion (Nagase et al., 2001). The flue gas, which contains CO₂ and NO, could provide not only a carbon source for microalgal growth but also an additional nitrogen source.

SO_x in flue gas is also an inhibitor of microalgal growth (Lee et al., 2000). The main form of SO_x in the flue gas generated from a coke oven is SO₂. Lee et al. (2000) reported that the growth of *Chlorella* KR-1 aerated with simulated flue gas containing 150 ppm SO₂ was suppressed because of cellular toxicity when a

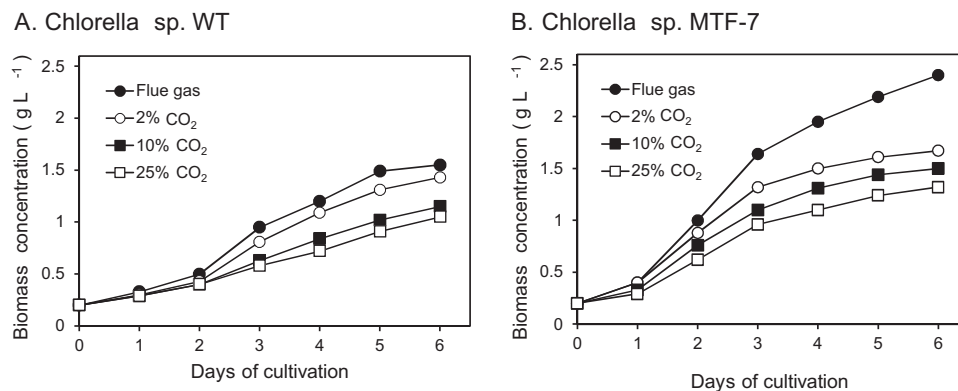


Fig. 1. Growth profiles of *Chlorella* sp. (wild-type, WT) (A) and its mutant, *Chlorella* sp. MTF-7 (B), cultured in an indoor photobioreactor aerated with continuous flue gas or CO₂-enriched gas (2%, 10%, or 25%). The initial biomass concentration was approximately 0.2 g L⁻¹. The microalgal cells were cultivated at ~300 μmol m⁻² s⁻¹. The flue gas was provided at 0.05 vvm. The cultures were grown for 6 days, and the microalgal cells were sampled every 24 h for growth determinations.

low-density initial biomass concentration (0.1 g L^{-1}) was used, but *Chlorella* KR-1 exhibited good growth when a high-density initial biomass concentration (0.5 g L^{-1}) was used. The toxic effect of SO_2 could be overcome by acidophilic microalgal isolation (Kurano et al., 1995; Lee et al., 2002). Hauck et al. (1996) reported that an acidophilic microalga, *Cyanidium caldarium*, grew well in the presence of 200 ppm SO_2 in simulated flue gas aeration. Considering that growth of most algal strains reported was completely inhibited, when the cultures aerated with flue gas which contained SO_2 concentration higher than 50 ppm (Kurano et al., 1995; Hauck et al., 1996). In our study, the isolated mutant strain, *Chlorella* sp. MTF-7, showed remarkably excellent tolerances to SO_2 and grew well in cultures supplied with gas containing approximately 90 ppm SO_2 when an initial biomass concentration of at least 0.5 g L^{-1} *Chlorella* sp. MTF-7 was used.

The satisfactory growth of *Chlorella* sp. MTF-7 in cultures supplied with gas containing approximately 90 ppm SO_2 may be due to its ability to tolerate highly oxidative molecular species. Bisulfite (HSO_3^-) and sulfite (SO_3^{2-}) are microalgal growth inhibitors that are formed in water from SO_2 (Yang et al., 2004). As SO_2 dissolves in the culture broth, HSO_3^- is formed: HSO_3^- can be converted to SO_3^{2-} and SO_4^{2-} at appropriate pH values. As HSO_3^- is converted to SO_4^{2-} , highly oxidative molecular species are formed, such as superoxide anions, hydrogen peroxide and hydroxyl radical. These highly oxidative molecular species can cause the peroxidation of membrane lipids and the bleaching of chlorophyll; thus, microalgal growth is inhibited by the processing of HSO_3^- to SO_4^{2-} (Ranieri et al., 1999; Noji et al., 2001). The inhibitory effect of SO_2 on *Chlorella* sp. MTF-7 growth might be eliminated by screening specific mutant strains and using a high concentration of inoculum.

To assess the potential of *Chlorella* sp. MTF-7 to be cultured by the side of the stack of a coke oven for the on-site bioremediation of flue gas without a cooling system, the growth of *Chlorella* sp. WT and *Chlorella* sp. MTF-7 when aerated with flue gas at different culture temperatures was also evaluated. Fig. 2 shows the growth curves of *Chlorella* sp. WT and *Chlorella* sp. MTF-7 when aerated with flue gas at 25, 30, 35 or 40 °C. The average growth rates of the *Chlorella* sp. WT cultures that were aerated with flue gas at 25, 30, 35 or 40 °C were 0.23, 0.21, 0.14 and $0.11 \text{ g L}^{-1} \text{ d}^{-1}$, respectively. The average growth rates of the *Chlorella* sp. MTF-7 cultures that were aerated with flue gas at 25, 30, 35 or 40 °C were 0.37, 0.39, 0.32 and $0.24 \text{ g L}^{-1} \text{ d}^{-1}$, respectively. The optimal growth temperature for *Chlorella* sp. MTF-7 was 30 °C, and the maximum biomass productivity of *Chlorella* sp. MTF-7 cultured at 30 °C and aerated with flue gas was $0.70 \text{ g L}^{-1} \text{ d}^{-1}$. However, the growth rate and biomass productivity of *Chlorella* sp. MTF-7 that was cultured

at higher temperatures (35 and 40 °C) remained high and were significantly greater than those of *Chlorella* sp. WT, even when the wild-type microalgal cells cultured at 25 and 30 °C.

3.1.2. Outdoor culture experiments

To evaluate microalgal growth performance during on-site flue gas aeration, a *Chlorella* sp. MTF-7 culture system was installed next to the smokestack of a coke oven at the China Steel Corporation in southern Taiwan (Supplementary Fig. S1). The flue gas from the coke oven was introduced into the microalgal cultures by suction pump, and air was supplied by an air pump. The gas was provided with either continuous flue gas aeration or intermittent flue gas aeration controlled by a gas-switching cycle operation (Supplementary Fig. S2). For continuous flue gas aeration, the flue gas was supplied continuously for 9 h during the day. For intermittent flue gas aeration, the flue gas was supplied in 30-min intervals every hour from 07:30 to 16:30; a gas-switching cycle was performed with a flue gas inlet load for 30 min followed by an air inlet load for 30 min (30 min flue gas/30 min air) for 9 h during the day.

Fig. 3 shows the growth curves that resulted when different initial biomass concentrations (0.5, 0.75, 1.0 and 1.25 g L^{-1}) of the *Chlorella* sp. MTF-7 inoculum were aerated with continuous (Fig. 3A) and intermittent flue gas (Fig. 3B) at 0.05 vvm. The growth profiles of *Chlorella* sp. MTF-7 aerated with flue gas were stable and linear with respect to the initial biomass concentration of the inoculum, whether the flue gas supply was continuous or intermittent. The average growth rates of *Chlorella* sp. MTF-7 when the initial biomass inoculum was 0.5, 0.75, 1.0 or 1.25 g L^{-1} were 0.13, 0.11, 0.11 and $0.05 \text{ g L}^{-1} \text{ d}^{-1}$ with continuous flue gas aeration, and 0.30, 0.36, 0.29 and $0.28 \text{ g L}^{-1} \text{ d}^{-1}$ with intermittent flue gas aeration, respectively. The growth rates of the cultures aerated with intermittent flue gas were 2.3-, 3.1-, 2.6- and 5.2-fold higher than those of the cultures aerated with continuous flue gas when initial biomass concentrations of 0.5, 0.75, 1.0 or 1.25 g L^{-1} were used, respectively. During a 6-day cultivation in which the initial biomass concentration of *Chlorella* sp. MTF-7 was 0.75 g L^{-1} , the maximum biomass growth rate was $0.52 \text{ g L}^{-1} \text{ d}^{-1}$, and the average biomass growth rate was $0.36 \text{ g L}^{-1} \text{ d}^{-1}$. The growth potential of *Chlorella* sp. MTF-7 cultures aerated with intermittent flue gas was significantly higher than that of *Chlorella* sp. MTF-7 cultures continuously aerated with flue gas. The intermittent flue gas aeration strategy for cultivation could enhance microalgal growth and also increase the utilization of the CO_2 in the flue gas. These results demonstrate that *Chlorella* sp. MTF-7 can grow well in an outdoor photobioreactor aerated directly with flue gas from a coke oven.

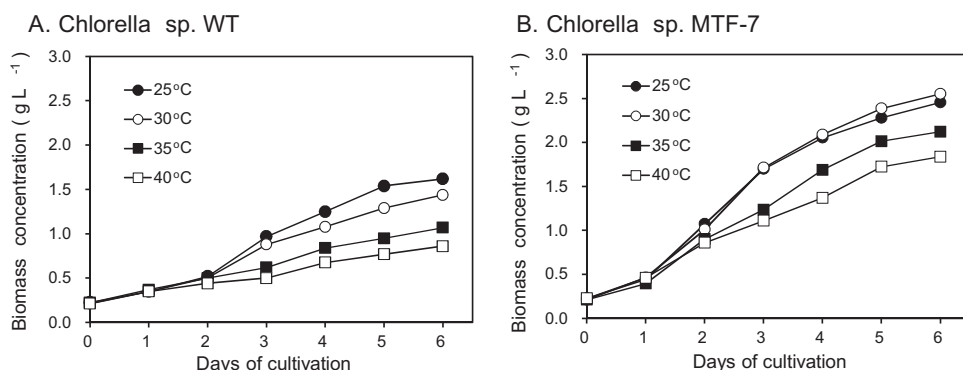
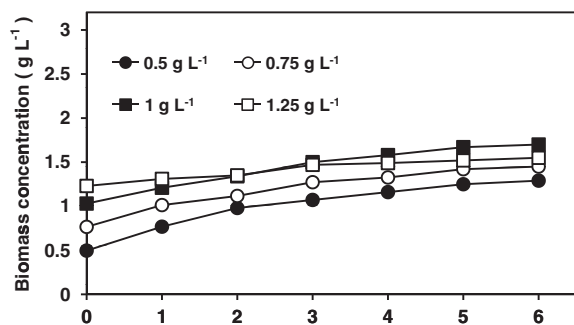


Fig. 2. Growth profiles of *Chlorella* sp. (wild-type, WT) (A) and its mutant, *Chlorella* sp. MTF-7 (B), cultured in an indoor photobioreactor aerated with continuous flue gas and operated at different temperatures (25, 30, 35 or 40 °C). The initial biomass concentration was approximately 0.2 g L^{-1} . The microalgal cells were cultivated at $\sim 300 \mu\text{mol m}^{-2} \text{ s}^{-1}$. The flue gas was provided at 0.05 vvm. The cultures were grown for 6 days, and the microalgal cells were sampled every 24 h for growth determinations.

A. Continuous flue gas aeration



B. Intermittent flue gas aeration

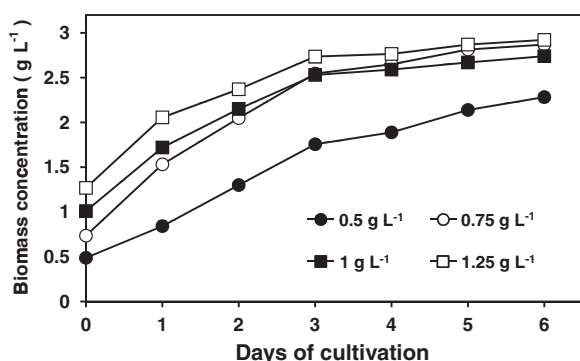


Fig. 3. Growth profiles of *Chlorella* sp. MTF-7 cultured in an outdoor photobioreactor aerated with continuous (A) or intermittent flue gas (B). The initial biomass concentrations were 0.5, 0.75, 1.0 and 1.25 g L⁻¹. The microalgal cells were cultivated during the day (1000 to 1800 $\mu\text{mol m}^{-2} \text{s}^{-1}$). The flue gas was provided at 0.05 vvm. The cultures were grown for 6 days, and the microalgal cells were sampled every 24 h for growth determinations.

3.2. Flue gas bioremediation by continuous flue gas aeration

3.2.1. CO₂ removal

For the on-site bioremediation of CO₂ in flue gas from a coke oven, the time course of the CO₂ removal efficiency of the microalgal cultures that were aerated with flue gas was determined. The CO₂ concentrations in the inlet and outlet loads of the flue gas used to aerate the microalgal cultures were monitored in real time by CO₂ gas sensors. Fig. 4A illustrates the inlet load, outlet load and CO₂ removal efficiency of *Chlorella* sp. MTF-7 under flue gas aeration. The CO₂ removal efficiency was 95% at 10 min after flue gas introduction and 50% after 25 min of flue gas aeration. The decrease in the CO₂ removal efficiency of the microalgal culture was due to the continuous inlet load of the flue gas. After aeration for 40 min, a constant 13% CO₂ removal efficiency was reached. Li et al. (2011) recently reported that the screened and isolated mutant *Scenedesmus obliquus* WUST4 could remove about 64% of the CO₂ from flue gas (18% v/v) generated from a coke oven. When a water pump was used to counter-circulate the water and increase the gas retention time, the efficiency of CO₂ removal from the flue gas by a microalgal culture reached 82.3 ± 12.5% (Vunjak-Novakovic et al., 2005). The result is also confirmed that according the regression lines of the relation between biomass concentration and the CO₂ removal efficiency, the CO₂ removal efficiency was approximate 20% (Chiu et al., 2009b). Moreover, this phenomenon is also confirmed in previous study that the CO₂ removal efficiency was remarkably consistent and showed a stable pattern from outlet CO₂ measurements (Chiu et al., 2008).

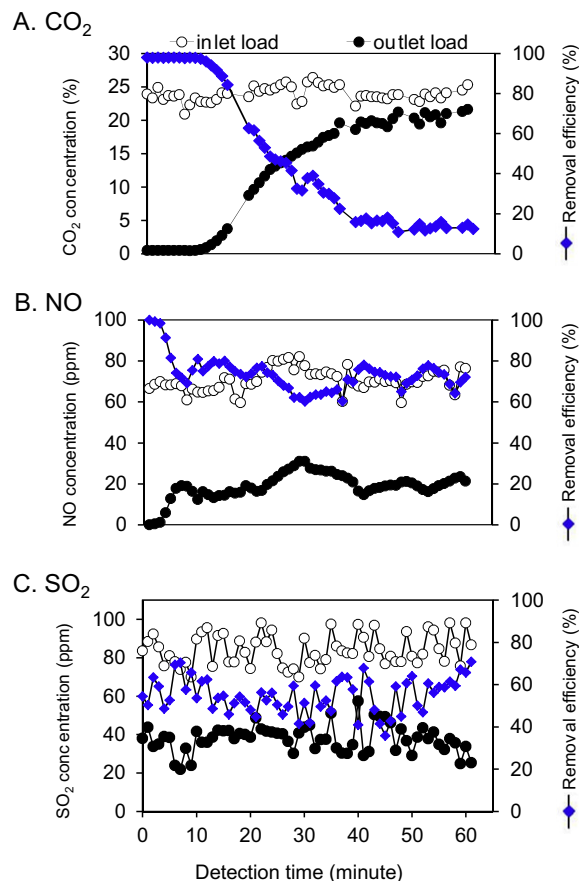


Fig. 4. The efficiency of CO₂ (A), NO (B) and SO₂ (C) removal from flue gas by *Chlorella* sp. MTF-7 cultures under continuous flue gas aeration. The biomass concentration of the microalgal culture was approximately 2 g L⁻¹, and it was cultivated during the day (1000 to 1800 $\mu\text{mol m}^{-2} \text{s}^{-1}$). The flue gas was provided under continuous operation at 0.05 vvm. The CO₂, NO and SO₂ concentration in the inlet load (open circle) and the outlet load (solid circle) of the flue gas was monitored in real time once every minute with a flue gas analyzer. The CO₂, NO and SO₂ removal efficiency (blue diamond) was calculated from the difference in the CO₂, NO and SO₂ concentrations of the inlet and outlet loads. (For interpretation of the references in color in this figure legend, the reader is referred to the web version of this article.)

3.2.2. NO and SO₂ removal

The time courses of the NO and SO₂ removal efficiencies of the microalgal cultures that were aerated with flue gas were also determined. Fig. 4B illustrates the inlet load, outlet load and NO removal efficiency of *Chlorella* sp. MTF-7 under flue gas aeration. The average NO concentrations of the inlet and outlet loads of the flue gas were 78 ± 4 and 20 ± 5 ppm. The NO removal efficiency of the *Chlorella* sp. MTF-7 cultures was approximately 73%. The SO₂ concentrations in the inlet and outlet loads of the flue gas were 87 ± 9 and 37 ± 7 ppm, respectively, and the SO₂ removal efficiency of the *Chlorella* sp. MTF-7 cultures under flue gas aeration was approximately 55% (Fig. 4C). Nagase et al. (2001) have reported that, in addition to reducing CO₂ in flue gas, microalgal cultures are effective continuous flue gas bioscrubbers for NO (>60% removal efficiency). Since most power stations should equipped with the flue gas desulfurization for SO_x emission control below 100 ppm, *Chlorella* sp. MTF-7 may be used for the direct CO₂, NO and SO₂ capture from the flue gases. To make the biological CO₂, NO and SO₂ capture process to be economically feasible, the utilization of the produced microalgal biomass is a critically important issue.

3.3. Flue gas bioremediation by a gas-switching cycle operation

3.3.1. CO₂ removal

To improve the efficiency of CO₂ removal from flue gas, a gas (flue-gas/air) -switching cycle operation was intermittently performed to introduce the flue gas into the microalgal cultures.

First, the CO₂ removal efficiency at different time intervals of intermittent flue gas aeration was evaluated. In the intermittent flue gas aeration, the culture aeration was controlled by a gas switch, and a gas-switching cycle was performed with a flue gas inlet load for 20, 30 or 40 min followed by an air inlet load for 30 min (i.e., 20, 30 or 40 min flue-gas/30 min air) during the day. The CO₂ concentrations of the inlet and outlet loads when *Chlorella* sp. MTF-7 was aerated with intermittent flue gas at 20, 30 or 40 min time intervals was illustrated (Supplementary Fig. S3). The average CO₂ removal efficiencies at the 20, 30 or 40 min time intervals were 70, 63 and 45%, respectively.

To evaluate the system stability for CO₂ removal during intermittent flue gas aeration, the intermittent flue gas aeration was operated with a 30-min time interval. CO₂ removal in the intermittent flue gas aeration of the *Chlorella* sp. MTF-7 culture was stable, and the inlet and outlet load patterns were also stable and similar to each other during the three gas-switching cycles (Fig. 5A). The pattern of the changes in the pH of the *Chlorella* sp. MTF-7 culture was stable during intermittent flue gas aeration (Fig. 5B). The pH value decreased from 7.9–8.2 to 6.3–6.5 after 30 min of flue gas aeration. After the gas was switched to air aeration, the pH value returned to 8.0 ± 0.2 after 30 min of air introduction. The fluctuations in the dissolved CO₂ in the microalgal culture broth also followed a repetitive pattern during the gas-switching aeration cycles (Fig. 5C). During flue gas aeration, the pH decreased, and the dissolved inorganic carbon (DIC) concentration increased because of CO₂ absorption. During air aeration, the pH increased, and the DIC concentration decreased, implying that the increase in the outlet load of CO₂ contributed to the pH decrease. Our system was operated for 9 cycles during the day, and the patterns of the fluctuations in the values of pH, DIC and CO₂ removal efficiency were stable throughout (data not shown).

3.3.2. NO and SO₂ removal

The NO and SO₂ removal efficiencies were also evaluated during the intermittent flue gas application. Fig. 6A illustrates the patterns of the NO concentrations of the inlet and outlet loads when *Chlorella* sp. MTF-7 was aerated with intermittent flue gas. The outlet load of NO was efficiently removed by the *Chlorella* sp. MTF-7 culture aerated with intermittent flue gas. The average NO removal efficiency reached approximately 70%. The patterns of the NO concentrations of the inlet and outlet loads were stable for each cycle of flue-gas/air switching. Fig. 6B illustrates the patterns of the SO₂ concentrations of the inlet and outlet loads when *Chlorella* sp. MTF-7 was aerated with intermittent flue gas. The SO₂ removal efficiency was also stable, and the average SO₂ removal efficiency reached approximately 50%.

To determine whether CO₂, NO and SO₂ in the flue gas could be continuously bioremediated with intermittent flue gas aeration, a double-set of photobioreactor system that alternately aerated with flue gas was established and operated (Supplementary Fig. S2). The flue gas aeration on/off time was controlled by a gas switch. There were two sets of gas switches. When the A gas switch was on for 30 min to allow flue gas aeration into system A, the B gas switch was off for 30 min to allow air aeration into system B. Therefore, flue gas was introduced into system A for 30 min and subsequently into system B for 30 min to permit continuous flue gas bioremediation. The inlet and outlet loads of the flue gas were monitored with a portable gas analyzer. According to calculations that were based on the CO₂, NO and SO₂ concentrations of the inlet and outlet loads, the

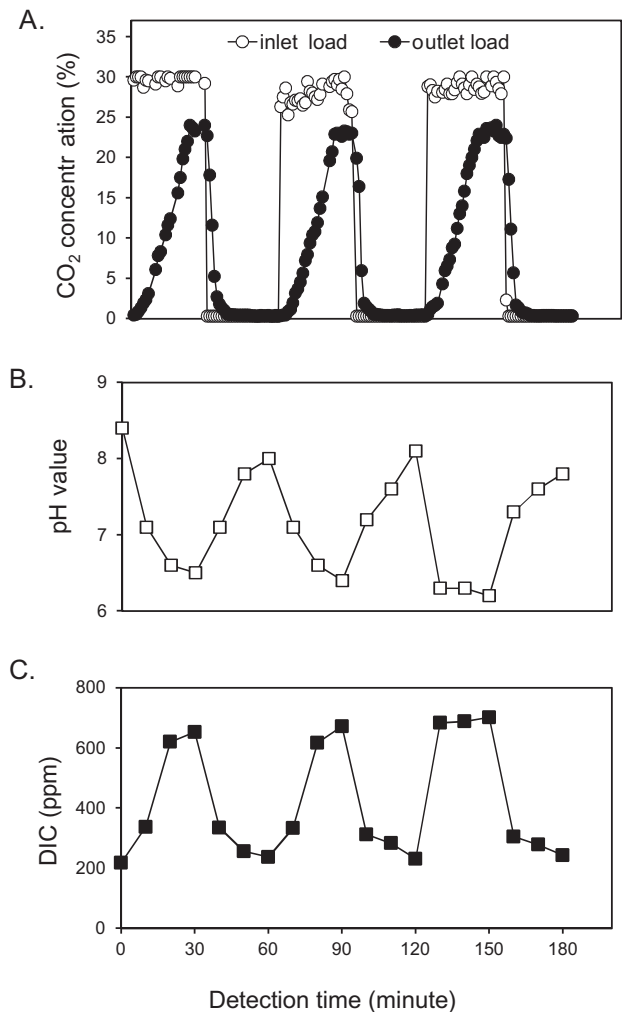


Fig. 5. The patterns of the inlet and outlet load CO₂ concentrations (A), pH value (B), and dissolved inorganic carbon (DIC) concentrations (C) of *Chlorella* sp. MTF-7 cultures aerated with intermittent flue gas at 0.05 vvm. The flue gas was controlled by a gas-switching cycle operation. A gas cycling switch was used with a flue gas inlet load for 30 min followed by an air inlet load for 30 min (30 min flue gas/30 min air). The CO₂ concentration was monitored at the inlet and outlet loads with a flue gas analyzer. The inlet and outlet loads of the gases were monitored once every minute. The culture broth was sampled every 10 min for the pH and DIC measurements.

average CO₂, NO and SO₂ removal efficiencies were maintained at 61, 68 and 51%, respectively. The gas-switching cycle operation was also extended to the double-set of photobioreactor system. This double-set of photobioreactor system was alternately aerated with flue gas. Via the gas-switching cycle operation, CO₂, NO and SO₂ could be constantly removed from the flue gas. The CO₂, NO and SO₂ removal efficiencies, respectively, were maintained at approximately 60%, 70% and 50% in the constant removal gas-switching cycle operation (data not shown). As mentioned above, *Chlorella* sp. MTF-7 cultures could be aerated directly with intermittent flue gas, and a strategy of gas-switching cycle without a pH control is a promising approach for continuous flue gas bioremediation. To achieve the desired flue gas bioremediation capacity, the photobioreactors could be multiplied and connected in parallel.

3.4. The effect of flue gas on lipid production in microalgal cells

To investigate the effects of flue gas aeration on lipid production in *Chlorella* sp. MTF-7, microalgal cells were harvested after 6 days

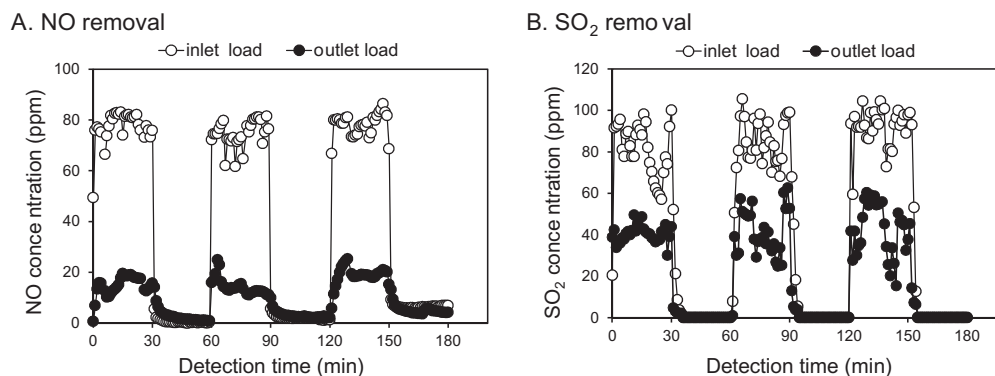


Fig. 6. The patterns of the inlet and outlet load NO (A) and SO₂ (B) concentrations of *Chlorella* sp. MTF-7 cultures aerated with intermittent flue gas at 0.05 vvm. The NO and SO₂ concentrations were monitored in real time at the inlet load (open circle) and the outlet load (solid circle) gas with a flue gas analyzer once every minute.

of flue gas and CO₂-enriched gas (25%) aeration. The lipid content of *Chlorella* sp. MTF-7 was $25.2 \pm 1.2\%$ ($n = 3$) when aerated with flue gas and $26.4 \pm 2.3\%$ ($n = 3$) when aerated with CO₂-enriched gas (25%). The lipid content was no significant difference between that of *Chlorella* sp. MTF-7 aerated with flue gas and CO₂-enriched gas. The fatty acid composition of the microalgal lipids was analyzed by GC. Supplementary Table 1 shows the main fatty acid compositions of *Chlorella* sp. MTF-7 aerated with CO₂-enriched gas or flue gas. Palmitic acid (C16:0) was the predominant fatty acid. Compared to the *Chlorella* sp. MTF-7 culture aerated with CO₂-enriched gas (25%), the *Chlorella* sp. MTF-7 culture aerated with flue gas had more palmitic acid (C16:0) ($55.2 \pm 4.5\%$ vs. $42.0 \pm 2.3\%$). In addition, the relative saturated fatty acid content of the *Chlorella* sp. MTF-7 culture aerated with the flue gas was also higher than that of the *Chlorella* sp. MTF-7 culture aerated with CO₂-enriched gas ($62.3 \pm 4.1\%$ vs. $48.6 \pm 2.7\%$).

Although the lipid content of *Chlorella* sp. MTF-7 aerated with flue gas was slightly lower than that of *Chlorella* sp. MTF-7 aerated with CO₂-enriched gas, the lipid productivity of the *Chlorella* sp. MTF-7 cultures aerated with flue gas was higher due to their higher growth rate. For the purpose of biodiesel production from algal biomass, lipid productivity should be a selection parameter and a critical variable for the evaluation of algal species and culture operation (Chiu et al., 2009a; Griffiths and Harrison, 2009; Chen et al., 2011). When compared with aeration with 25% CO₂-enriched gas, aeration with flue gas had no significant effect on the lipid content of *Chlorella* sp. MTF-7 cultures. Our results suggest that a *Chlorella* sp. MTF-7 culture aerated with flue gas is a suitable candidate for microalgal lipid production. The most common fatty esters in biodiesel are palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3) (Knothe, 2008). The fatty acid profile of *Chlorella* sp. MTF-7 consists mainly of C₁₆ and C₁₈ fatty acids, and the major composition of the fatty acids 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). The fatty acids of *Chlorella* sp. MTF-7 are mainly saturated. The saturated fatty acid content in *Chlorella* sp. MTF-7 aerated with flue gas was higher than that of *Chlorella* sp. MTF-7 aerated with enriched CO₂. The properties of a biodiesel fuel, including its ignition quality, combustion heat, cold filter plugging point (CFPP), oxidative stability, viscosity, and lubricity, are determined by the structure of its component fatty esters. High levels of saturated fatty acids tend to increase the stability of biodiesel because unsaturated fatty acids result in poor oxidative stability. The effect of flue gas on fatty acid compositions was not clear and would be further investigated.

4. Conclusion

A thermal- and CO₂-tolerant mutant strain, *Chlorella* sp. MTF-7, can act as a bioscrubber to directly and efficiently remove CO₂, NO and SO₂ from flue gas. The microalgal cultures operated by intermittent flue gas aeration, growth potential of the microalgal strain could be enhanced, and CO₂, NO and SO₂ in the flue gas were efficiently removed. We have also demonstrated a practical operation in which the flue gas from coke oven of steel plant directly supplied the carbon source for biomass production of the microalgal strain.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biortech.2011.06.091.

References

- Béchet, Q., Shilton, A., Oliver, B., Fringer, O.B., Muñoz, R., Guieysse, B., 2010. Mechanistic modeling of broth temperature in outdoor photobioreactors. *Environ. Sci. Technol.* 44, 2197–2203.
- Brennan, L., Owende, P., 2010. Biofuels from microalgae – A review of technologies for production, processing, and extractions of biofuels and co-products. *Renew. Sust. Energ. Rev.* 14, 557–577.
- Brune, D.E., Lundquist, T.J., Benemann, J.R., 2009. Microalgal biomass for greenhouse gas reductions: potential for replacement of fossil fuels and animal feeds. *J. Environ. Eng. ASCE* 135, 1136–1144.
- Chen, C.Y., Yeh, K.L., Aisyah, R., Lee, D.J., Chang, J.S., 2011. Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: a critical review. *Bioresour. Technol.* 102, 71–81.
- Chiu, S.Y., Kao, C.Y., Chen, C.H., Kuan, T.C., Ong, S.C., Lin, C.S., 2008. Reduction of CO₂ by a high-density culture of *Chlorella* sp. in a semicontinuous photobioreactor. *Bioresour. Technol.* 99, 3389–3396.
- Chiu, S.Y., Kao, C.Y., Tsai, M.T., Ong, S.C., Chen, C.H., Lin, C.S., 2009a. Lipid accumulation and CO₂ utilization of *Nannochloropsis oculata* in response to CO₂ aeration. *Bioresour. Technol.* 100, 833–838.
- Chiu, S.Y., Tsai, M.T., Kao, C.Y., Ong, S.C., Chen, C.H., Lin, C.S., 2009b. The air-lift photobioreactors with flow patterning for a high-density culture of microalgae and carbon dioxide removal. *Eng. Life Sci.* 9, 254–260.
- Doucha, J., Straka, F., Livinsky, K., 2005. Utilization of flue gas for cultivation of microalgae *Chlorella* sp. in an outdoor open thin-layer photobioreactor. *J. Appl. Phycol.* 17, 403–412.

- Douskova, I., Doucha, J., Livansky, K., Machat, J., Novak, P., Umysova, D., Zachleder, V., Vitova, M., 2009. Simultaneous flue gas bioremediation and reduction of microalgal biomass production costs. *Appl. Microbiol. Biotechnol.* 82, 179–185.
- Favre, E., Bounaceur, R., Roizard, D., 2009. Biogas, membranes and carbon dioxide capture. *J. Membr. Sci.* 238, 11–14.
- Gielen, D., 2003. CO₂ removal in the iron and steel industry. *Energy Conv. Manag.* 44, 1027–1037.
- Griffiths, M.J., Harrison, T.L., 2009. Lipid productivity as a key characteristic for choosing algal species for biodiesel production. *J. Appl. Phycol.* 21, 493–507.
- Hauck, J.T., Olson, G.J., Scierka, S.J., Perry, M.D., Atai, M., 1996. In 212th ACS National Meeting, vol. 41(4), Orlando, FL, pp. 1391–1396.
- Ho, S.H., Chen, C.Y., Lee, D.J., Chang, J.S., 2011. Perspectives on microalgal CO₂-emission mitigation systems – A review. *Biotechnol. Adv.* 29, 189–198.
- Kadam, K.L., 2002. Environmental implications of power generation via coal-microalgae cofiring. *Energy* 27, 905–922.
- Knothe, G., 2008. Designer biodiesel: optimizing fatty ester composition to improve fuel properties. *Energy Fuels* 22, 1358–1364.
- Kumar, K., Dasgupta, C.N., Nayak, B., Lindblad, P., Das, D., 2011. Development of suitable photobioreactors for CO₂ sequestration addressing global warming using green algae and cyanobacteria. *Bioresour. Technol.* 102, 4945–4953.
- Kurano, N., Ikemoto, H., Miyashita, H., Hasegawa, T., Hata, H., Miyachi, S., 1995. Fixation and utilization of carbon dioxide by microalgal photosynthesis. *Energy Conv. Manag.* 36, 689–692.
- Lee, J.S., Lee, J.P., 2003. Review of advances in biological CO₂ mitigation technology. *Biotechnol. Bioprocess. Eng.* 8, 354–359.
- Lee, J.N., Lee, J.S., Shin, C.S., Park, S.C., Kim, S.W., 2000. Methods to enhance tolerances of *Chlorella* KR-1 to toxic compounds in flue gas. *Appl. Biochem. Biotechnol.* 84 (86), 329–342.
- Lee, J.S., Kim, D.K., Lee, J.P., Park, S.C., Koh, J.H., Cho, H.S., Kim, S.W., 2002. Effects of SO₂ and NO on growth of *Chlorella* sp. KR-1. *Bioresour. Technol.* 82, 1–4.
- Lee, J.Y., Yoo, C., Jun, S.Y., Ahn, C.Y., Oh, H.M., 2010. Comparison of several methods for effective lipid extraction from microalgae. *Bioresour. Technol.* 101, S75–S77.
- Li, F., Yang, Z., Zeng, R., Yang, G., Chang, X., Yan, J., Hou, Y., 2011. Microalgae capture of CO₂ from actual flue gas discharged from a combustion chamber. *Ind. Eng. Chem. Res.* Accepted doi:10.1021/ie200040q.
- Mata, T.M., Martins, A.A., Caetano, N.S., 2010. Microalgae for biodiesel production and other applications: a review. *Renew. Sust. Energ. Rev.* 14, 217–232.
- Nagase, H., Yoshihara, K., Eguchi, K., Okamoto, Y., Murasaki, S., Yamashita, R., Hirata, K., Miyamoto, K., 2001. Uptake pathway and continuous removal of nitric oxide from flue gas using microalgae. *Biochem. Eng. J.* 7, 241–246.
- Negoro, M., Shioji, N., Miyamoto, K., Miura, Y., 1999. Growth of microalgae in high CO₂ gas and effects of SO_x and NO_x. *Appl. Biochem. Biotechnol.* 28, 877–886.
- Noji, M., Saito, M., Nakamura, M., Aono, M., Saji, H., Saito, K., 2001. Cysteine synthase overexpression in tobacco confers tolerance to sulfur-containing environmental pollutants. *Plant Physiol.* 126, 973–980.
- Ong, S.C., Kao, C.Y., Chiu, S.Y., Tsai, M.T., Lin, C.S., 2010. Characterization of the thermal-tolerant mutants of *Chlorella* sp. with high growth rate and application on outdoor photobioreactor cultivation. *Bioresour. Technol.* 101, 2880–2883.
- Packer, M., 2009. Algal capture of carbon dioxide; biomass generation as a tool for greenhouse gas mitigation with reference to New Zealand energy strategy and policy. *Energy Policy* 3428, 3437.
- Patil, V., Kallqvist, T., Olsen, E., Vogt, G., Gislerod, H.R., 2007. Fatty acid composition of 12 microalgae for possible use in aquaculture feed. *Aquac. Int.* 15, 1–9.
- Ranieri, A., Pieruccetti, F., Castagna, A., Lorenzini, G., Soldatin, G.F., 1999. SO₂-induced decrease in photosynthetic activity in two barley cultivars. Evidence against specific damage at the protein-pigment complex level. *Plant Physiol.* Biochem. 37, 919–929.
- Vunjak-Novakovic, G., Kim, Y., Wu, X., Berzin, I., Merchuk, J.C., 2005. Air-lift bioreactors for algal growth on flue gas: mathematical modeling and pilot-plant studies. *Ind. Eng. Chem. Res.* 44, 6154–6163.
- Wang, B., Li, Y., Wu, N., Lan, C.Q., 2008. CO₂ bio-mitigation using microalgae. *Appl. Microbiol. Biotechnol.* 79, 707–718.
- Yang, S., Wang, J., Cong, W., Cai, Z., Ouyang, F., 2004. Effects of bisulfite and sulfite on the microalga *Botryococcus braunii*. *Enzyme Microb. Technol.* 35, 46–50.
- Yoo, C., Jun, S.Y., Lee, J.Y., Ahn, C.Y., Oh, H.M., 2010. Selection of microalgae for lipid production under high levels carbon dioxide. *Bioresour. Technol.* 101, S71–S74.