國 立 交 通 大 學

生 物 科 技 學 院

生 物 科 技 學 系

博士論文

建構微藻光生物反應系統並用於二氧化碳減量與微藻生物質的生產

Establishing a Microalgae-incorporated Photobioreactor System for CO_2 Reduction and Microalgal Biomass Production

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建構微藻光生物反應系統並用於二氧化碳減量與微藻生物質的生產

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摘　要

以光合生物反應器培養微藻可被用來減量廢氣中的二氧化碳（CO_2），且微藻所生產之油脂還可被轉化為生質柴油使用。在本研究中，我們篩選並分離出具有高生長潛能之微藻細胞來減量 CO_2 及生產微藻生物質。此外我們也設計一氣舉式光合生物反應器用以進行微藻的高濃度培養。

首先，我們以 CO_2 減量並生產生物質為目的進行微藻細胞株之篩選，分離得到具有高生長潛能之藻株，再以起始藻細胞濃度為低濃度(i.e., 8×10^5 cells mL^{-1})與高濃度(i.e., 8×10^6 cells mL^{-1})的培養方式來進行微藻二氧化碳耐受性試驗，試驗結果顯示當微藻之起始細胞濃度較高時，在具有二氧化碳通入培養的環境下，微藻生長速率會較為快速，由上述結果可知微藻對於 CO_2 的耐受能力會因藻細胞濃度的增加而增加，因此本研究將於微藻培養的起始階段，通入適當的 CO_2 (2%)，使藻細胞適應通有 CO_2 之環境下生長，再配合半連續式的培養方式將微藻轉至 10%及 15% CO_2 環境下培養，使微藻細胞能逐漸適應高濃度 CO_2，進而克服高濃度 CO_2 對於微藻生長之抑制，提昇微藻對於 CO_2 之耐受性。

接著，為了增加生物質生產與 CO_2 移除之效率，我們以半連續式之各種培養策略進行生物質產能評估，每兩天置換四分之一培養液、每三天置換三分之一培養液及每八天置換二分之一培養液，此半連續式培養結果顯示，以每兩天置換四分之一之培養，微藻生物質產能可高達 0.61 g L^{-1} d^{-1}，於本研究中所設計之多孔內管氣舉式光生物反應器不但具有高生物質產能且能維持高密度培養，同時我們也評估多孔內管氣舉式光生物反應器對於 CO_2 之移除效能。由結果顯示，高濃度微藻(5 g L^{-1})培養於通氣為 10% CO_2 的環境下，CO_2 之移除效能大於 60%以上。

最後，我們篩選了一耐溫與耐受 CO_2 微藻突變株 *Chlorella* sp. MTF-7 實際應用於實

場煙道廢氣養殖。研究結果顯示，我們所篩選之 *Chlorella* sp. MTF-7 於室內不同溫度以中鋼煙道廢氣通氣實驗中，*Chlorella* sp. MTF-7 於 35°C 及 40°C 之高溫下，微藻生物質產能則為 0.32 及 0.24 g L^{-1} d^{-1}；直接引中鋼煙道廢氣進行戶外 *Chlorella* sp. MTF-7 養殖六天，微藻養殖濃度可達 2.87 g L^{-1}（起始養殖濃度為 0.75 g L^{-1}），微藻生物質產能則為 0.52 g L^{-1} d^{-1}。經由兩組間歇煙道廢氣通氣方式進行養殖，其 CO_2、NO 及 SO_2 之移除效能分別約為 60%、70%及 50%。由結果顯示，所篩選得之 *Chlorella* sp. MTF-7 可有效直接利用煙道廢氣養殖，並能穩定生產微藻生物質與有效減除煙道廢氣中之 CO_2、NO 及 SO_2。

關鍵字： 煙道廢氣、二氧化碳、微藻、生物質、光生物反應器、小球藻

Establishing a Microalgae-incorporated Photobioreactor System for CO_2 Reduction and Microalgal Biomass Production

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Abstract

Microalgal cultivated in photobioreactor can be used for CO_2 mitigation from waste gas and microalgal lipids can be converted into biodiesel. In this study, we screened and isolated microalgal strains with high potential for CO_2 reduction and microalgal biomass production. In addition, we also designed an air-lift photobioreactor for high density microalgal cultivation.

First, the high growth potential microalgal cells were screened and isolated as a candidate for CO_2 reduction and biomass production. Then, the low (i.e., 8×10^5 cells mL^{-1}) and high (i.e., 8×10^6 cells mL^{-1}) density of the microalgal cells inoculums for CO_2 tolerance was evaluated. The results indicate that microalgal cells grew rapidly in a high-density culture with CO_2 aeration. Thus, the strategy of increasing CO_2 tolerance and cell density in the microalgal cultures was performed in this study. At the initiating stage of culture, the microalgal cells were grown and adapted to an enriched-CO_2 (2%) environment. Then, the semicontinuous system was performed. The result shows that the microalgal cells can grow well even under the conditions of 10% and 15% CO_2 aeration.

Then, for increasing biomass production and CO_2 removal efficiency, the microalgal cells cultivated in the operation mode that culture broth was replaced by 1/4 (i.e., one-fourth volume of cultured broth was replaced by fresh medium at an interval of 2 days) and 1/3 (one-third broth replaced at 3 days interval) and 1/2 (one-second broth replaced at 8 days interval). The results show that the maximum biomass productivity could achieve 0.61 g L^{-1} d^{-1} in 1/4 of the culture broth recovered from the culture every 2 days. The CO_2 removal efficiency was also evaluated because the high performance of biomass production and high density cultivation. The results show that > 60% of CO_2 could be removed from the aerated gas which contains 10% CO_2 under high density (approximate 5 g L^{-1}) cultivation.

Finally, the growth and on-site bioremediation potential of an isolated thermal- and

CO_2-tolerant mutant strain, *Chlorella* sp. MTF-7, were investigated. The biomass productivity of *Chlorella* sp. MTF-7 cultured indoors at 35 and 40°C was 0.32 and 0.24 g L^{-1} d^{-1}, respectively. The *Chlorella* sp. MTF-7 cultures were directly aerated with the flue gas generated from coke oven of a steel plant. The biomass concentration, productivity 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}. By the operation with intermittent flue gas aeration in a double-set photobioreactor system, average efficiency of CO_2 removal from the flue gas could reach to 60%, and NO and SO_2 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 CO_2, NO and SO_2 from flue gas simultaneously.

Keyword: flue gas, carbon dioxide, microalga, biomass, photobioreactor, *Chlorella* sp.

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Figure 21. The patterns of the inlet and outlet load NO (**A**) and SO_2 (**B**) concentrations of *Chlorella* sp. MTF-7 cultures aerated with intermittent flue gas at 0.05 vvm. **110110**

1. Introduction

1.1 General Introduction

Global warming, which is induced by increasing concentrations of greenhouse gases in the atmosphere, is of great concern. Carbon dioxide (CO_2) is the principal greenhouse gas and the concentration of atmospheric CO_2 has increased rapidly since the onset of industrialization. The global atmospheric concentration of CO_2 has increased from about 280 ppm of a pre-industrial value to 387 ppm in 2010 (NOAA, 2010). Such an increasing long-lived greenhouse gas currently makes the Earth heating. And now the drastic climate change induced by increasing concentrations of greenhouse gas in the atmosphere is also of great concern. Furthermore, the drastic energy crisis is due to the shortage of oil and additionally to electricity or other natural resources. There are several means of reducing the emissions of greenhouse gases by energy production from renewable sources. This issue has received increasing attention due to the exhaustion of natural sources of fossil fuels (Favre et al., 2009).

There are various research strategies on CO_2 sequestration which have been carried out, such as physical, chemical and biological methods. The example of physical method is geological storage of CO_2, which it is injected into reservoirs (Packer, 2009). On the other hand, the examples chemical methods are by washing with alkaline solutions (Diao et al., 2004), multiwalled carbon nanotubes (Su et al., 2009), amine coating activated carbon (Plaza et al., 2007) for CO_2 capture in order to reduce the emission of CO_2. The biological method using microalgal photosynthesis is considered as an effective approach for biological CO_2 fixation (de Morais and Costa, 2007a; Wang et al., 2008; González López et al., 2009; Packer, 2009; Yoo et al., 2010). By the biological approaches, CO_2 can be fixed into microalgal biomass via photosynthesis. Microalgae has about 10 – 50 times higher CO_2 fixation rates than terrestrial plants and can thus utilize CO_2 from flue gas to produce biomass efficiently (Doucha et al., 2005, Wang et al., 2008; Brune et al., 2009). Therefore, reduction of the emission from industries or power plants by the use of microalga incorporated photobioreactor is a potential method for directly removing CO_2 from waste gas.

In addition, microalgae can use CO_2 efficiently and accumulate lipids, which are chemically similar to common vegetable oils and are high potential sources for biodiesel production. Compared with fossil fuels, the microalgal-based biodiesel are renewable, biodegradable, and low pollutant produced. Thus, reducing atmospheric CO_2 by microalgal

photosynthesis is considered safe and reliable for living environment. A closed photobioreactor can be a bioscrubber for waste gas treatment and the microalgal cells cultured in the photobioreactor converting CO_2 from the waste gas into biomass is energy-efficient and economical approach.

Flue gases from power plant are responsible for more than 7% of the world CO_2 emissions from energy use and steel plants are the single largest source of energy-related CO_2 emissions in the world. In general, the primary emission in flue gas is CO_2. This CO_2 is a plentiful carbon source for microalgal cultures. The direct use of the flue gas reduces the cost of pretreatment. However, the direct use of the flue gas 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. Temperature is also an inhibitory growth factor for outdoor microalgal cultivation.

Even though that the microalgae have the widen areas of characterization, the microalgae have to be improved to select more potential strains which show the potential of biomass production and remove more CO_2. For on-site bioremediation of CO_2, NO_X and SO_X from flue gas, first, we aimed to characterize the effect of CO_2 on biomass production. There are lots of researches and applications on the large scale outdoor photobioreactor cultivation system for the potential in CO_2 reduction. However, there are limited literatures reported about on-site bioremediation by microalgal cultures and the performance of outdoor enclosed photobioreactors still can not even achieve the values obtained at laboratory scale. Thus, we aimed to isolate thermal- and flue gas tolerant mutant strains, which can grow well in an outdoor closed photobioreactor for bioremediation under strong sunlight without the supply of cooling system in the area of subtropical region.

1.2 Microalgae

Microalgae covers all unicellular and simple multi-cellular microorganisms, including both prokaryotic microalgae and eukaryotic microalgae (Scott et al., 2010). Algae can either be autotrophic or heterotrophic; the former require only inorganic compounds such as CO_2, salts and a light energy source for growth; while the latter are non-photosynthetic therefore require an external source of organic compounds as well as nutrients as an energy source. For autotrophic algae, photosynthesis is a key component of their survival, whereby they convert solar radiation and CO_2 absorbed by chloroplasts into adenosine triphosphate (ATP) and O_2

the usable energy currency at cellular level, which is then used in respiration to produce energy to support growth (Falkowski and Raven, 1997).

Microalgae, just like plants, are photosynthetic microorganisms which convert sunlight, CO_2 and water to biomass, potential biofuels, foods, feeds and high-value bioactives (Borowitzka, 1999; Banerjee et al., 2002; Walter et al., 2005; Spolaore et al., 2006; Chisti, 2007). Microalgae are responsible for over 50% of primary photosynthetic productivity on earth and are budding sunlight factories for a wide range of potentially useful products, but are scarcely used commercially (Gavrilescu and Chisti, 2005; Wijffels, 2007). The large-scale cultivation of microalgae and the use of its biomass for the production of useful products were first considered seriously in Germany during World War II (Becker, 1994).

Microalgae have the potential to develop biotechnology in a number of areas including nutrition, aquaculture, pharmaceuticals, and biofuels. Microalgae produce many valuable substances such as vitamins and color pigments, essential fatty acids, amino acids, and even antibiotics and pharmaceutically-active substances, such as high-quality food, food supplements or alternatives for synthetic substances in the cosmetics and chemical industry. Microalgae has the wide range of benefits in producing valuable chemicals or healthy foods, vitamins and as feedstock for animals on land and in aquaculture (Pulz and Gross, 2004; Spolaore et al., 2006; Raja et al., 2008), consume waste and the metallic pollutants in wastewater (Perales-Vela et al., 2006; Jácome-Pilco et al., 2008) and produce biodiesel (Chisti, 2007; Hossain et al., 2008). Microalgae convert CO_2 into biomass and use CO_2 efficiently. Therefore, microalgae are cultivated at large-scale outdoor for the purpose of industrialization.

In the reason of depleting supplies and the contribution of petroleum or natural fossil fuels to the accumulation of CO_2 in the environment, continued use of these fuels is now widely recognized as unsustainable. Renewable, carbon neutral, transport fuels are necessary for environmental and economic sustainability. Biodiesel (monoalkyl esters) is nontoxic and less emissions of CO_2, sulfur oxides (SO_X) and nitrogen oxides (NO_X), and it is biodegradable and renewable as well as environmentally safe (Ma and Hanna, 1999). Biodiesel derived from oil crops is a potential renewable and carbon neutral alternative to petroleum fuels. Unfortunately, biodiesel from oil crops, waste cooking oil and animal fat cannot realistically satisfy even a small fraction of the existing demand for transport fuels. Microalgae appear to be the only source of renewable biodiesel that is capable of meeting the global demand for transport fuels. Like plants, microalgae use sunlight to produce oils but they do so more

efficiently than crop plants. Oil productivity of many microalgae greatly exceeds the oil productivity of the best producing oil crops (Chisti, 2007). **Table 1** shows comparison of microalgae with other biodiesel feedstocks. Moreover, microalgal biomass can be used to produce biofuel by pyrolysis, direct combustion or thermal chemical liquefaction (Mata et al., 2010). The lipid fraction of microalgal biomass can be extracted and transesterified for biodiesel production (Li et. al, 2008; Brennan and Owende, 2010; Lee et al., 2010).

Although algae have been commercially cultivated for over 50 years, metabolic engineering now seems necessary in order to achieve their full processing capabilities. Recently, the development of a number of transgenic algal strains boasting recombinant protein expression, engineered photosynthesis, and enhanced metabolism encourage the prospects of designer microalgae (Rosenberg et al., 2008).

1.2.1 *Chlorella*

Chlorella species are encountered in all water habitats exhibiting a cosmopolitan occurrence, having been isolated from widely differing fresh, as well as marine, water habitats. The species of the genus *Chlorella* have simple life cycles and nutritional requirements. Classification is complex because *Chlorella* species cannot be readily discerned on the basis of morphological features, the taxonomy of *Euchlorella*, which comprises the most common species is, therefore, incomplete. It has indeed been proposed to use physiological and biochemical rather than morphological criteria, for species identification. On the basis of their external morphlogy, *Chlorella* species could nevertheless be placed in four general groups: (1) spherical cells (ratio of the two axes equals one); (2) ellipsoidal cells (ratio of the longest axis to the shortest axis being 1.45 to 1.60); (3) spherical or ellipsoidal cells; (4) globular to subspherical cells (Richmond, 1986). In reproduction, which is exclusively asexual, each mature cell divides usually producing four or eight (and more rarely, 16) autospores, which are freed by rupture or dissolution of the parental walls.

1.2.2 Microalgal Physiology

Autotrophic organisms obtain their energy through the absorption of light energy for the reduction of CO_2 by the oxidation of substrate, mainly water, with the release of O_2. Photoautotrophic organisms only require inorganic mineral ions and obligate photoautotrophs

are those that cannot grow in the dark. By far, most algae belong to this category, although many require minimal quantities of organic compounds for growth, such as vitamins.

For high rates of autotrophic production, supply of CO_2 and HCO_3^- is most important. Contrary to land plants, atmospheric CO_2 cannot satisfy the C-requirements of high yielding autotrophic algal production systems. The CO_2-H_2CO_3- HCO_3-CO_3^{2-} system is the most important buffer generally present in culture broth and it is the best means available to control and maintain specific pH levels that are optimal for mass-cultivated species. The bicarbonate-carbonate buffer system can provide CO_2 for photosynthesis. The buffer system reactions imply that during photosynthetic CO_2 fixation, OH^- accumulates in the growth solution leading to a gradual rise in pH. pH-static control via direct CO_2 sparging into the culture media is the best and most convenient method for pH control and at the same time supplying CO_2 for high yield in mass algal cultures. Since active photosynthesis results in an increase in pH, the opposite is true for CO_2 release during respiration. The overall influence is little since as a general rule dark respiration is less than 10% of total photosynthetic production (Grobbelaar and Soeder, 1985).

After carbon, nitrogen is the most important nutrient contributing to the biomass produced. The nitrogen content of the biomass can range from 1% to more than 10% and it not only varies between different groups but within a particular species, depending on the supply and availability. Typical responses to nitrogen limitation is discoloration of the cells and accumulation of organic carbon compound such as polysaccharides and certain oils (Becker, 1994). Nitrogen is mostly supplied as nitrate (NO_3^-), but often ammonia (NH_4^+) and urea are also used, with similar growth rates recorded. Ammonia nitrogen is often the preferred N-source for microorganisms and the assimilation of either NO_3^- or NH_4^+ is related to the pH of the growth media. When ammonia is used as the sole source of N, the pH could drop significantly during active growth, due to the release of H^+ ions. An increase in pH occurs when deciding whether to supply either nitrate or ammonia, is that the latter could be lost form the growth media due to volatilization, particularly when the pH increases.

Sulfur is generally present in small quantity in all plant cells but is probably not a limiting factor for many algae under normal conditions. Sulfur is incorporated into numerous organic compounds and sulfates are present in the vacuoles. As compared with other macronutrients sulfur uptake and metabolism in algae have been studied only scarcely. In fact, major studies on sulfur assimilation by algae were done more than 20 years ago and present research in this field equals nearly nothing. Uptake sulfur by both *Chlorella pyrenoidosa* and

Scenedesmus sp. is stimulated by light (Kylin, 1961; Tseng et al., 1971). As with N assimilation, light could be acting by providing energy via photophosphorylation, reductant, or C skeletons. A large part of the sulfur in most algae is incorporated into protein. Two sulfur-containing amino acids, cysteine and methionine, are important in maintaining the three-dimensional configuration of proteins through sulfur bridges. Incorporation of sulfur from sulfate in the medium into normal cells of *Scenedesmus* was enhanced by light relatively most in the case of lipid S and least in the inorganic sulfate fraction; the effects of light were, generally, increased by the presence of CO_2 and nitrogen salts.

1.3 Microalgal Cultivation

Most microalgae are strictly photosynthetic, i.e., they need light and carbon dioxide as energy and carbon sources. Photoautotrophic production is the only method which is technically and economically feasible for large-scale production of algae biomass (Borowitzka, 1997). Two systems that have been widely constructed are based on open pond and closed photobioreactor technologies (Borowitzka, 1999). The technical viability of each system is influenced by intrinsic properties of the selected algae strain used, as well as climatic conditions and the costs of land and water (Borowitzka, 1992). Microalgae cultivation using sunlight energy can be carried out in open ponds or closed photobioreactors, based on tubular, flat plate, or other designs. Closed systems are much more expensive than ponds, present significant operating challenges, such as gas exchange limitations, cannot be scaled up much beyond about 100 m^2 for an individual growth unit. **Table 2** shows the advantages and disadvantages of open and closed culture systems. Currently there are three types of industrial reactors used for algal culture: (1) open ponds, (2), photobioreactors and (3) closed and hybrid systems.

Open-pond systems are shallow ponds in which algae are cultivated. Nutrients can be provided through runoff water from nearby land areas or by channeling the water from sewage/water treatment plants. Technical and biological limitations of the open systems have given rise to the development of enclosed photobioreactors. Photobioreactors are different types of tanks or closed systems in which algae are cultivated. Microalgae cultivation using sunlight energy can be carried out in open ponds or closed photobioreactors, based on tubular, flat plate, or other designs. The closed systems have been considered to be capital intensive and are justified only when a fine chemical is to be produced. Microalgae production in closed photobioreactors is highly expensive. Closed systems are much more expensive than

ponds. However, closed systems require much less light and agricultural land to grow algae (Chisti, 2007). As much as 25% of the biomass produced during daylight may be lost during the night due to respiration. The extent of this loss depends on the light level under which the biomass was grown, the growth temperature, and the temperature at night (Chisti, 2007). Algal cultures consist of a single or several specific strains optimized for producing the desired product. Water, necessary nutrients, and CO_2 are provided in a controlled way, while oxygen has to be removed (Carlsson et al., 2007). Algae receive sunlight either directly through the transparent container walls or via light fibers or tubes that channel it from sunlight collectors. A great amount of developmental work to optimize different photobioreactor systems for algae cultivation has been carried out (Carvalho et al., 2006, Choi et al., 2003, Hankamer et al., 2007, Janssen et al., 2003).

Photobioreactors are the preferred method for scientific researchers, and recently for some newer, innovative production designs. These systems are more expensive to build and operate; however, they allow for a very controlled environment. This means that gas levels, temperature, pH, mixing, media concentration, and light can be optimized for maximum production (Chisti 2007). Unlike open ponds, Photobioreactors can ensure a single alga species is grown without interference or competition (Campbell 2008).

1.4 Open Pond System

Open ponds are the oldest and simplest systems for mass cultivation of microalgae. The pond is designed in a raceway configuration, in which a paddlewheel circulates and mixes the algal cells and nutrients. The raceways are typically made from poured concrete, or they are simply dug into the earth and lined with a plastic liner to prevent the ground from soaking up the liquid. Baffles in the channel guide the flow around the bends in order to minimize space. Algal cultures can be defined (one or more selected strains), or are made up of an undefined mixture of strains. The only practicable methods of large-scale production of microalgae are raceway ponds (Terry and Raymond 1985; Molina Grima 1999) and tubular photobioreactors (Molina Grima et al., 1999; Tredici 1999; Sánchez Mirón et al., 1999). Open architecture approaches, while possibly the cheapest of all current techniques, suffer challenges with contamination, evaporation, temperature control, CO_2 utilization, and maintainability. The ponds are kept shallow because of the need to keep the algae exposed to sunlight and the limited depth to which sunlight can penetrate the pond water. The ponds are operated continuously; that is, water and nutrients are constantly fed to the pond while algae-containing

water is removed at the other end. Large-scale outdoor culture of microalgae and cyanobacteria in open ponds, raceways, and lagoons is well established (Becker 1994). Open culture is used commercially in the USA, Japan, Australia, India, Thailand, China, Israel, and elsewhere to produce algae for food, feed, and extraction of metabolites. Open-culture systems allow relatively inexpensive production but are subject to contamination. Consequently, only a few algal species can be cultured in open outdoor systems. Species that grow successfully in the open include rapid growers such as *Chlorella* and species that require a highly selective extremophilic environment that does not favor the growth of most potential contaminants. For example, species such as *Spirulina* and *Dunaliella* thrive in highly alkaline and saline selective environments, respectively. Algae produced in quantities in open systems include *Spirulina*, *Chlorella*, *Dunaliella*, *Haematococcus*, *Anabaena*, and *Nostoc* (Chisti 2006). The size of these ponds is measured in terms of surface area, since surface area is so critical to capturing sunlight. Even at levels of productivity that would stretch the limits of an aggressive research and development program, such systems require acres of land. At such large sizes, it is more appropriate to think of these operations on the scale of a farm. Such algae farms would be based on the use of open, shallow ponds in which some source of waste CO_2 could be efficiently bubbled into the ponds and captured by the algae. Careful control of pH and other physical conditions for introducing CO_2 into the ponds allows for more than 90% utilization of injected CO_2. Raceway ponds, usually lined with plastic or cement, are about 15 to 35 cm deep to ensure adequate exposure to sunlight. Paddlewheels provide motive force and keep the algae suspended in the water. The ponds are supplied with water and nutrients, and mature algae are continuously removed at one end. A raceway pond is made up of a closed-loop recirculation channel that is typically about 0.3 m deep. Flow is guided around bends by baffles placed in the flow channel, and raceway channels are built in concrete or compacted earth and may be lined with white plastic. During daylight, the culture is fed continuously in front of the paddlewheel where the flow begins. Broth is harvested behind the paddlewheel on completion of the circulation loop. The paddlewheel operates all the time to prevent sedimentation. Photosynthesis is the most important biochemical process in which plants, algae, and some bacteria harness the energy of sunlight to produce food. Productivity is affected by contamination with unwanted algae and microorganisms that feed on algae. Open ponds, specifically mixed raceway ponds, are much cheaper to build and operate, can be scaled up to several hectares for individual ponds, and are the method of choice for commercial microalgae production. However, such open ponds also suffer from various limitations, including more rapid (than closed systems) biological invasions by other

algae, algae grazers, fungi and amoeba, etc., and temperature limitations in cold or hot humid climates. Microalgae can be cultivated in coastal areas. The raceway pond system of biomass culture must be approved to achieve high and sustained growth rates and oil yields that are essential to developing an algal-based biofuel industry.

1.5 Photobioreactor

Photobioreactors are different types of tanks or closed systems in which algae are cultivated (Richmond, 2004). Photobioreactors have the ability to produce algae while performing beneficial tasks, such as scrubbing power plant flue gases or removing nutrients from wastewater (Carlsson et al., 2007). Microalgal biomass can play an important role in solving the problem between the production of food and that of biofuels in the near future. Phototropic microalgae are most commonly grown in open ponds and photobioreactors (Patil et al., 2005). Photobioreactors offer a closed culture environment, which is protected from direct fallout and so is relatively safe from invading microorganisms. This technology is relatively expensive compared to the open ponds because of the infrastructure costs. An ideal biomass production system should use the freely available sunlight. Many different designs of photobioreactor have been developed, but a tubular photobioreactor seems to be most satisfactory for producing algal biomass on the scale needed for biofuel production (Tredici, 1999). Closed, controlled, indoor algal photobioreactors driven by artificial light are already economical for special high-value products such as pharmaceuticals, which can be combined with the production of biodiesel to reduce the cost (Patil et al., 2008).

Photobioreactors have higher efficiency and biomass concentration (2 to 5 g/L), shorter harvest time (2 to 4 weeks), and higher surface-to-volume ratio than open ponds (Lee, 2001; Wang et al., 2008). Closed systems consist of numerous designs: tubular, flat-plated, rectangular, continued stirred reactors. The typical closed photobioreactors are demonstrated in **Figure 1**. Photobioreactors in general provide better control of cultivation conditions, yield higher productivity and reproducibility, reduce contamination risk, and allow greater selection of algal species used for cultivation. The photobioreactor has a photolimited central dark zone and a better lighting peripheral zone close to the surface (Chisti, 2007). CO_2-enriched air is sparged into the photobioreactor creating a turbulent flow. Turbulent flow simultaneously circulates cultures between the light and dark zones and assists the mass transfer of CO_2 and O_2 gases. The frequency of light and dark zone cycling is depended on the intensity of turbulence, cell concentration, optical properties of the culture, tube diameter, and the external

irradiance level (Chisti, 2007). The highest cost for closed systems is the energy cost associated with the mixing mechanism (Wijffels 2008). Tubular photobioreactors consist of transparent tubes that are made of flexible plastic or glass. Tubes can be arranged vertically, horizontally, inclined, helically, or in a horizontal thin-panel design. Tubes are generally placed in parallel to each other or flat above the ground to maximize the illumination surface-to-volume ratio of the reactor. The diameter of tubes is usually small and limited (0.2-m diameter or less) to allow light penetration to the center of the tube where the linear growth rate of culture decrease with increasing unit diameter (Ogbonna and Tanaka 1997). The growth medium circulates from a reservoir to the reactor and back to the reservoir. A turbulent flow is maintained in the photobioreactor to ensure distribution of nutrients, improve gas exchange, minimize cell sedimentation, and circulate biomass for equal illumination between the light and dark zones. The tubes are generally less than 10 cm in diameter to maximize sunlight penetration. The medium broth is circulated through a pump to the tubes, where it is exposed to light for photosynthesis, and then back to a reservoir. A portion of the algae is usually harvested after it passes through the solar collection tubes, making continuous algal culture possible. In some photobioreactors, the tubes are coiled spirals to form what is known as a helical-tubular photobioreactor. The microalgal broth is circulated from a reservoir to the solar collector and back to the reservoir (Chisti 2007). Flat-plated photobioreactors are usually made of transparent material. The large illumination surface area allows high photosynthetic efficiency, low accumulation of dissolved O_2 concentration, and immobilization of algae (Ugwu et al., 2008). The photobioreactors are inexpensive and easy to construct and maintain. However, the large surface area presents scale-up problems, including difficulties in controlling culture temperature and carbon dioxide diffusion rate and the tendency for algae adhering to the walls. Vertical-column photobioreactors are compact, low-cost, and easy to operate monoseptically. Furthermore, they are very promising for large-scale cultivation of algae. It was reported that bubble-column and airlift photobioreactors (up to 0.19 m in diameter) can attain a final biomass concentration and specific growth rate that are comparable to values typically reported for narrow tubular photobioreactors (Sánchez Mirón et al., 2002). Some bubble column photobioreactors are equipped with either draft tubes or constructed as split cylinders. In the case of draft tube photobioreactors, intermixing occurs between the riser and the downcomer zones of the photobioreactor through the walls of the draft tube (Chiu et al., 2009b).

1.6 Closed Hybrid System

Other system designs for algae production are possible. The Japanese, French, and German governments have invested significant R&D dollars on novel closed photobioreactor designs for algae production. The main advantage of such closed systems is that they are not as subject to contamination. When designing a photobioreactor, design parameters such as reactor dimension, flowrate, light requirements, culture condition, algae species, reproducibility, and economic value need to be taken into consideration. Depending on the reactor dimensions, site location, and local climate, these parameters can determine the type of cultivation system needed (open versus closed). Reactor design should have good mixing properties, efficiency, and reproducibility and be easy to maintain and sterilize. An efficient photobioreactor not only improves productivity but also is used to cultivate multiple strains of algae. The performance of a photobioreactor is measured by volumetric productivity, areal productivity, and productivity per unit of illuminated surface (Riesing, 2006). Volumetric productivity is a function of biomass concentration per unit volume of bioreactor per unit of time. Areal productivity is defined as biomass concentration per unit of occupied land per unit of time. Productivity per unit of illuminated surface is measured as biomass concentration per area per unit of time. Closed photobioreactors support up to fivefold higher productivity with respect to reactor volume and consequently have a smaller "footprint" on a yield basis. Besides saving water, energy, and chemicals, closed photobioreactors have many other advantages that are increasingly making them the reactor of choice for biofuel production, as their costs are lower (Schenk et al., 2008). Closed photobioreactors permit essentially single-species culture of microalgae for prolonged periods. Most closed photobioreactors are designed as tubular reactors, plate reactors, or bubble column photobioreactors (Pulz, 2001). Other less common designs like semihollow spheres have been reported to run successfully (Sato et al., 2006). Closed photobioreactors have been employed to overcome the contamination and evaporation problems encountered in open ponds (Molina Grima et al., 1999). These systems are made of transparent materials and are generally placed outdoors for illumination by natural light. The cultivation vessels have a large surface-area to-volume ratio. The main problems in the large-scale cultivation of microalgae outdoors in open ponds are low productivity and contamination. To overcome these problems, a closed system consisting of polyethylenes sleeves was developed. The closed system was found to be superior to open ponds with respect to growth and production in a number of microalgae. In both closed and open systems, growth and production under continuous operation were higher than in batch

cultivation (Cohen et al., 1991). The preferred alternative is closed photobioreactors, where the algae fluid remains in a closed environment to enable accelerated growth and better control over environmental conditions. These glass or plastic enclosures, often operated under modest pressure, can be mounted in a variety of horizontal or vertical configurations and can take many different shapes and sizes. Rigid frameworks or structures are usually used to support the photobioreactor enclosures.

In hybrid systems, both open ponds as well as closed photobioreactor system are used in combination to get better results. Open ponds are a very proficient and lucrative method of cultivating algae, but they become contaminated with superfluous species very quickly. A combination of both systems is probably the most logical choice for cost-effective cultivation of high yielding strains for biofuels. Open ponds are inoculated with a desired strain that had invariably been cultivated in a photobioreactor, whether it is as simple as a plastic bag or a high-tech fiber-optic bioreactor. Importantly, the size of the inoculums needs to be large enough for the desired species to establish in the open system before an unwanted species. Therefore, to minimize contamination issues, cleaning or flushing the ponds should be part of the aquaculture routine, and as such, open ponds can be considered as batch cultures (Schenk et al., 2008). Abundant light, which is necessary for photosynthesis, is the third requirement. This is often accomplished by situating the facility in a geographic location with abundant, uninterrupted sunshine (Brown and Zeiler 1993). This is a favored approach when cultivating in open ponds. Photobioreactors are flexible systems that can be optimized according to the biological and physiological characteristics of the algal species being cultivated, allowing one to cultivate algal species that cannot be grown in open ponds. A great proportion of light does not impose directly on the culture surface but has to cross the transparent photobioreactor walls.

In spite of their advantages, it is not expected that photobioreactor have a significant impact in the near future on any product or process that can be attained in large outdoor raceway ponds. Photobioreactors suffer from several drawbacks that need to be considered and solved. Their main limitations include: difficulty in scaling up, the high cost of building, operating and of algal biomass cultivation, overheating, bio-fouling, oxygen accumulation, and cell damage by shear stress and deterioration of material used for the photo-stage.
The cost of biomass production in photobioreactors may be one order of magnitude higher than in ponds. Whereas in some cases, for some microalgae species and applications it may be low enough to be attractive for aquaculture use; in other cases, the higher cell concentration and the higher productivity achieved in photobioreactor may not balance for its higher capital

and operating costs.

1.7 Factors influencing the growth of algae

Numerous aspects influence the growth and lipid content of algae. The reaction driving the initial conversion of sunlight into stored energy is photosynthesis. Therefore, all of the components involved in photosynthesis contribute to growth. The major factors include lighting, mixing, CO_2 enrichment, O_2 removal, nutrient supply, temperature, and pH (Suh and Lee, 2003a; Richmond, 2004b; Carvalho et al., 2006; Grobbelaar, 2009). It is important to note that in each category the precise conditions for optimal growth depend on the strain of algae selected for cultivation.

1.7.1 Light supply

An optimal reactor enhances light intensity/ penetration, as well as the wavelength of light and the frequency of cellular exposure to light. When selecting the light source, both the spectral quality and intensity must be considered. The spectral quality of light utilized by algae is defined by the absorption spectrum in the range of 400 to 700 nm for the chlorophyll and other photosynthetically active pigments, and the algal photosynthesis efficiency is a function of the spectral quality of the light source (Simmer et al., 1994; Suh and Lee, 2003a).

The level of light intensity is critical because at a certain level algae experience light saturation and dissipate the excess energy as heat (Mussgnug et al., 2007). Light saturation can be mitigated by the spatial dilution of light, which is the distribution of solar radiation on a greater photosynthetic surface are, and also reduces mutual shading of microalgal cells. Thus, a design principle for photobioreactor designs is to maximize the surface area to volume ratio, which can be used for comparison between reactors.

Beyond the surface area and volume, the unique geometry of a reactor influences the light distribution. For example in a tubular reactor, the light gradient is primarily determined by the diameter of the tube and the biomass density in the medium (Janssen et al., 2003). Optimal cell density is specific to each strain and needs to be maintained in order for light intensity and light penetration to remain at optimal levels (Richmond, 2004b). Light and dark cycles strongly influence the growth of algae. In both open ponds and outdoor closed reactors, natural light is subject to changes in time of day, weather, season, and geography (Pulz and Scheinbenbogen, 1998). Unfortunately, all reactors using natural light are subject to the

absence of light during nighttime. According to Chisti (2007), biomass losses might reach as high as 25% during the night, depending on the light intensity during the day, the temperature during the day, and the temperature at night. Janssen et al. (2003) stated that the length of the light/dark cycles experienced by algae influenced photosynthetic efficiency.

1.7.2 Mixing

The level of mixing in a reactor strongly contributes to the growth of algae. When environmental conditions do not limit growth rates, mixing is the most influential factor contributing to algae growth rates (Suh and Lee, 2003a). Mixing affects growth in two primary ways: (1) improves productivity by increasing the frequency of cell exposure to light and dark volumes of the reactor and, (2) by increasing mass transfer between the nutrients and cells (Qiang and Richmond, 1996).

Mixing and lighting are closely related, as mixing is often responsible for inducing the light and dark cycles beneficial to algae growth. Similarly, mixing offers little benefit if lighting is poor and or culture in low density (Richmond, 2004b). Ugwu et al. (2005) demonstrated that the installation of static mixers in tubular reactors succeeded in increasing light utilization and biomass yields when the reactor was scaled up by increasing the tube diameter.

1.7.3 Carbon dioxide consumption

In addition to light and water, CO_2 is necessary for photosynthesis to occur. However, an excess of CO_2 can also be detrimental to photosynthesis and cell growth. CO_2 concentrations from 1 to 5% (by volume) often lead to maximum growth. Despite this, laboratories routinely aerate algal cultures with 5 – 15% CO_2, or even pure CO_2 (Suh and Lee, 2003a).

Flue gas is a desirable source of CO_2 because it reduces greenhouse gas emissions as well as the cost of algal biofuel production. Flue gas from typical coal-fired power plants contain up to 13% CO_2 (Chisti, 2007). Doucha et al. (2005) studied the performance of a closed reactor utilizing flue gas as a source of CO_2 versus a reactor utilizing pure CO_2. Surprisingly, productivities and photosynthetic efficiencies were very similar under conditions of pure CO_2 versus flue gas. Because CO_2 concentration in flue gas was relatively low, the efficiency of CO_2 mass transfer was lower for flue gas than it was for pure CO_2.

1.7.4 Oxygen removal

A high presence of oxygen (O_2) around algae cells is undesirable. The combination of

intense sunlight and high oxygen concentration results in photooxidative damage to algal cells (Chisti, 2007).

Because of the constraint on the concentration of dissolved oxygen, tube length is limited in horizontal tubular reactors. This restriction makes it very difficult for tubular reactors to be scaled-up. In a tubular reactor designed by Molina et al. (2001), the algae culture regularly returned to an airlift zone where the accumulated oxygen from photosynthesis was stripped by air. A gas-liquid separator in the upper part of the airlift column prevented gas bubbles from recirculating into the horizontal loop of the airlift reactor. The time taken by the fluid to travel the length of the degasser must at least equal the time required by the oxygen bubbles to rise out.

1.7.5 Nutrient supply

In order to grow, algae require more than the reactants in the photosynthesis reaction. Two major nutrients are nitrogen and phosphorus, which both play a role in controlling growth rates. Other essential nutrients are carbon, hydrogen, oxygen, sulfur, calcium, magnesium, sodium, potassium, and chlorine. Nutrients needed in minute quantities include iron, boron, manganese, copper, molybdenum, vanadium, cobalt, nickel, silicon, and selenium (Suh and Lee, 2003a).

1.7.6 Temperature

Temperatures experienced by algae grown outdoors can vary as much as the extreme outdoor temperatures characteristic to the geographic region of cultivation. Although algae may be able to grow at a variety of temperatures, optimal growth is limited to a narrow range specific to each strain. Seasonal and even daily fluctuations in temperature can interfere with algae production. Temperatures can reach as high as 30℃ higher than ambient temperature in a closed photobioreactor without temperature control equipment (Suh and Lee, 2003a). Evaporate cooling, water spray or shading techniques are employed frequently to inhibit temperatures of that magnitude. Whereas, a lower temperature appears to reduce the loss of biomass due to respiration during the night (Chisti, 2007).

1.7.7 pH

Each strain of algae also has a narrow optimal range of pH. The pH of the medium is linked to the concentration of CO_2. Suh and Lee (2003a) mentioned that pH increases steadily in the medium as CO_2 is consumed during flow downstream in a reactor. The pH affects the liquid chemistry of polar compounds and the availability of nutrients such as iron, organic

acids, and even CO_2 (Lee and Pirt, 1984). Because pH is so influential, Suh and Lee (2003a) stated that commercial pH controllers must be used in reactors to optimize growth.

1.8 CO_2 Reduction by Microalgal Cultures

Generally, microalgae can typically capture CO_2 from three different sources: atmosphere, emission from power plants and industrial processes, and from soluble carbonate (Wang et al., 2008). CO_2 capture from atmosphere is probably the most basic method to sink carbon, and relies on the mass transfer from the air to the microalgae in their aquatic growth environments during photosynthesis.

There are three main CO_2 mitigation strategies are normally used, (1) physical method, (2) chemical reaction-based approaches, and (3) the biological mitigation. Most of carbon capture and sequestration (CCS) discussions are about geological storage of CO_2 presently. Whilst the oil and gas industry has successfully injected CO_2 into reservoirs, just before date this has mainly been for increased yield of fossil hydrocarbon reserves and not for long-term storage. This is proven safe but the biggest difficulty with this approach is the added cost of separation of CO_2 from the emission streams (Packer, 2009). The chemical reaction-based CO_2 mitigation approaches are energy-consuming, use costly processes, and have disposal problems because both the captured CO_2 and the wasted absorbents need to be disposed of. In other hand, the biological CO_2 mitigation has attracted much attention in the last years since it leads to the production of biomass energy in the process of CO_2 fixation through photosynthesis (Pulz and Gross, 2004).

A number of microalgae species are able to assimilate CO_2 from soluble carbonates such as sodium carbonate (Na_2CO_3) and sodium bicarbonate ($NaHCO_3$). Due to the high salt content and resulting high pH of the medium, it is easier to control invasive species since only a very small number of algae can grow in the extreme conditions (Colman and Rotatore, 1995; Emma et al., 2000; Wang et. al., 2008). The selection of suitable microalgae strains for CO_2 bio-mitigation has significant effect on efficacy and cost competitiveness of the bio-mitigation process. The desirable characteristics for high CO_2 fixation include: high growth and CO_2 utilisation rates; high tolerance of trace constituents of flue gases such as SOx and NOx; possibility for valuable by-products and co-products, e.g. biodiesel and biomass for solid fuels; ease of harvesting associated with spontaneous settling or bio-flocculation characteristics; high water temperature tolerance to minimize cost of cooling exhaust flue gases; be able to

use the strain in conjunction with wastewater treatment.

For example, de Morais and Costa (2007a) using *Spirulina* sp., obtained a maximum daily CO_2 biofixation of 53.29% for 6% (v/v) CO_2 and 45.61% for 12% (v/v) CO_2 in the injected flue gas, with the highest mean fixation rate being 37.9% for 6% (v/v) CO_2. With *Scenedesmus obliquus*, de Morais and Costa achieved biofixation rates of 28.08% and 13.56% for 6% (v/v) and 12% (v/v) CO_2, respectively.

Chang and Yang (2003) found that certain species of *Chlorella* could grow in an atmosphere containing CO_2 up to 40% (v/v). When comparing *Botryococcus braunii*, *Chlorella vulgaris* and *Scenedesmus* sp. under flue gas conditions, Yoo et al. (2010) found *Scenedesmus* sp. to be the most suitable for CO_2 mitigation due to high rates of biomass production (0.218 g L^{-1} d^{-1}). *B. braunii* and *Scenedesmus* sp. were found to grow better using flue gas as compared to air enhanced with CO_2. This is similar with an earlier study by Brown (1996) who found that microalgae can tolerant with flue gas very well.

In contrast, CO_2 capture from flue gas emissions from power plants that burn fossil fuels achieves better recovery due to the higher CO_2 concentration of up to 20% (Bilanovic et al., 2009), and adaptability of this process for both photobioreactor and raceway pond systems for microalgae production (Brennan and Owende, 2010). Flue gases from power plants are responsible for more than 7% of the total world CO_2 emissions from energy use (Kadam, 1997). Also, industrial exhaust gases contains up to 15% CO_2 (Maeda et al., 1995; Kadam, 2001), providing a CO_2-rich source for microalgae cultivation and a potentially more efficient route for CO_2 bio-fixation. **Table 3** summarized the productivity of biomass grown outdoors in the various photobioreactors. These outdoor cultivation showed the potential of microalgal cultivation for on-site bioremediation of CO_2 from flue gas.

In order to have an optimal yield, these algae need to have CO_2 in large quantities in the basins or bioreactors where they grow. Thus, the photobioreactors need to be coupled with traditional electricity-producing thermal power centers that produce CO_2 at an average rate of 13% of the total flue gas emissions. The CO_2 is put into the photobioreactor and assimilated by the algae. Outdoor microalgal culture coupled with flue gas aeration is with economic value and potential strategy for large-scale microalgal cultivation.

GreenFuel Technologies, one of the earliest, best funded and most publicized algae companies was a startup that developed a process of growing algae using emissions from fossil fuel, mainly to produce biofuel from algae. A beta emission reduction system was

installed at an MIT cogeneration facility in 2004 and after performing beyond expectations was moved to a larger power plant in fall 2005. Pilot units were tested at power plants in Arizona, Massachusetts and New York. Although the algal biomass produced by the process consists of proteins, lipids and carbohydrates which could be used to produce a variety of products, GreenFuel Technologies seems to be focusing on biofuel products. GreenFuel's large scale algae to biofuel process at the Arizona Public Service Redhawk power facility won the 2006 Platts Emissions Energy Project of the Year Award.

In 2007, the company had to shut down its third-generation bioreactor facility in Arizona after the plant produced more algae than the company's equipment could handle. At the same time, the company learned that its algae harvesting system would cost twice as much as expected. Though GreenFuel Technologies finally shut down operations on May 13, 2009, GreenFuel Technologies was the frontier of industrialized process of microalgal cultivation and microalgal bioremediation. At present time, there are many microalgal industries for microalgal cultivation process and they also focus on microalgal bioenergy. **Table 4** list the main international companies that using microalgal cultivation for CO_2 reduction and bioerergy development.

1.9 Potential of Biodiesel Produced from Microalgae

There are four important potential benefits of algae biomass cultivation that other sources don't have. First, algae biomass can be produced at extremely high volumes, and this biomass can yield a much higher percentage of oil than other sources. Second, algal oil has limited market competition. Third, algae can be cultivated on marginal land, fresh water, or sea water. Fourth, innovations to algae production allow it to become more productive while consuming resources that would otherwise be considered waste (Campbell, 2008). Biodiesel derived from oil crops is a potential renewable and carbon-neutral alternative to petroleum fuels. Unfortunately, biodiesel from oil crops, waste cooking oil, and animal fat cannot realistically satisfy even a small fraction of the existing demand for transport fuels. Microalgae appear to be the only source of renewable biodiesel that is capable of meeting the global demand for transport fuels.

Like plants, microalgae use sunlight to produce oils, but they do so more efficiently than crop plants. Oil productivity of many microalgae greatly exceeds the oil productivity of the best producing oil crops. Approaches to making microalgal biodiesel economically competitive with petrodiesel have been discussed (Chisti, 2007). Biodiesel from microalgae

seems to be the only renewable biofuel that has the potential to completely displace petroleum-derived transport fuels without adversely affecting the food supply and other crop products. Most productive oil crops, such as oil palm, do not come close to microalgae in being able to sustainably provide the necessary amounts of biodiesel. Similarly, bioethanol from sugar cane is no match for microalgal biodiesel (Chisti, 2008). According to some estimates, the yield of oil from algae is over 200 times the yield from the best performing plant/vegetable oils (Sheehan et al., 1998). The production of algae to harvest oil for biodiesel has not been undertaken on a commercial scale, but working feasibility studies have been conducted to arrive. Algae, like all plants, require large quantities of nitrogen fertilizer and water, plus significant fossil energy inputs for the functioning system (Goldman and Ryther, 1977). Harvesting the algae from tanks and separating the oil from the algae are difficult and energy-intensive processes (Pimentel et al., 2004; Pimentel, 2008).

1.10 Microalgae in Other Applications

Commercial large-scale production of microalgae started in the early 1960s in Japan with the culture of *Chlorella* as a food additive, which was followed in the 1970s and 1980s by expanded world production in countries such as USA, India, Israel, and Australia (Pulz and Scheinbenbogen, 1998; Borowitzka, 1999; Spolaore et al., 2006).

1.10.1 Health food and human food

The human consumption of microalgae biomass is limited to very few species due to the strict food safety regulations (Pulz and Gross, 2004), commercial factors, market demand and specific preparation. Among the abundant species of microalgae, *Chlorella*, *Spirulina* and *Dunaliella* dominate the market. Microalgae biomass is marketed in tablet or powder form as food additives generally in the health food market, which is expected to remain a stable market (Spolaore et al., 2006).

Chlorella is also used for medicinal value such as protection against renal failure and growth promotion of intestinal lactobacillus (Yamaguchi, 1996). Suggested health benefits including efficacy on gastric ulcers, wounds and constipation together with preventive action against both atherosclerosis and hyper-cholesterol and antitumor activity. *Dunaliella salina*, is exploited for its β-carotene content of up to 14% (Metting, 1996).*Spirulina* (*Arthrospira*) is used in human nutrition because of it high protein content and excellent nutrient value (Spolaore et al., 2006). Many companies have been producing ‘‘nutraceuticals’’ (food

supplements with claimed nutritional and medicinal benefits) made from *Spirulina*, such as in DIC in Japan and China, and Cyanotech in Kona and Hawaii.

1.10.2 Animal feed and aquaculture

Microalgae are an important food source and feed additive in the commercial rearing of many aquatic animals (Borowitzka, 2006). Over 30% of the current world algal production is sold for animal feed and over 50% of the world production of *Spirulina* is used as feed supplements (Spolaore et al., 2006).

Specific algal species are suitable for preparation of animal feed supplements. Algae species such as *Chlorella*, *Scenedesmus* and *Spirulina* have beneficial aspects including improved immune response, improved fertility, better weight control, healthier skin and a lustrous coat (Pulz and Gross, 2004). However, prolonged feeding at high concentrations could be detrimental (Spolaore et al., 2006) especially in relation to cyanobacteria.

1.10.3 Carotenoids and astaxanthin

Algae contain carotenoids, yellow orange or red pigments, that include β-carotene a substance converted by the body to Vitamin A. The most important uses of carotenoids are as food colorants and as supplements for human and animal feeds (Becker, 1994; Spolaore et al., 2006).

Astaxanthin is another carotenoid that can be derived from algae, *Haematococcus*, and is principally used in fish farming and as a dietary supplement or antioxidant. Natural astaxanthin is preferred for example in carp, chicken and red sea bream diets due to enhanced natural pigment deposition, regulatory requirements and consumer demand for natural products (Spolaore et al., 2006).

1.10.4 Polyunsaturated fatty acids

Polyunsaturated fatty acids (PUFAs) are essential for human development and physiology (Hu et al., 2008), and have been proven to reduce the risk of cardiovascular disease (Ruxton et al., 2007). Currently, fish and fish oil are the main sources of PUFA but application as a food additive are limited due to possible accumulation of toxins, fish odor, unpleasant taste, poor oxidative stability, the presence of mixed fatty acids and not suitable for vegetarian diets (Pulz and Gross, 2004).

Microalgal PUFA has many other applications such as additives for infant milk formula. Elsewhere, chickens have been fed with special algae to produce omega-3 enriched eggs (Pulz

and Gross, 2004). Currently, docosahexaenoic acid (DHA) is the only algal PUFA that is commercially available, because algal extracts are still not competitive sources of eicosapentaenoic acid (EPA), γ-linolenic acid (GLA), and arachidonic acid (AA) against other primary sources (Spolaore et al., 2006).

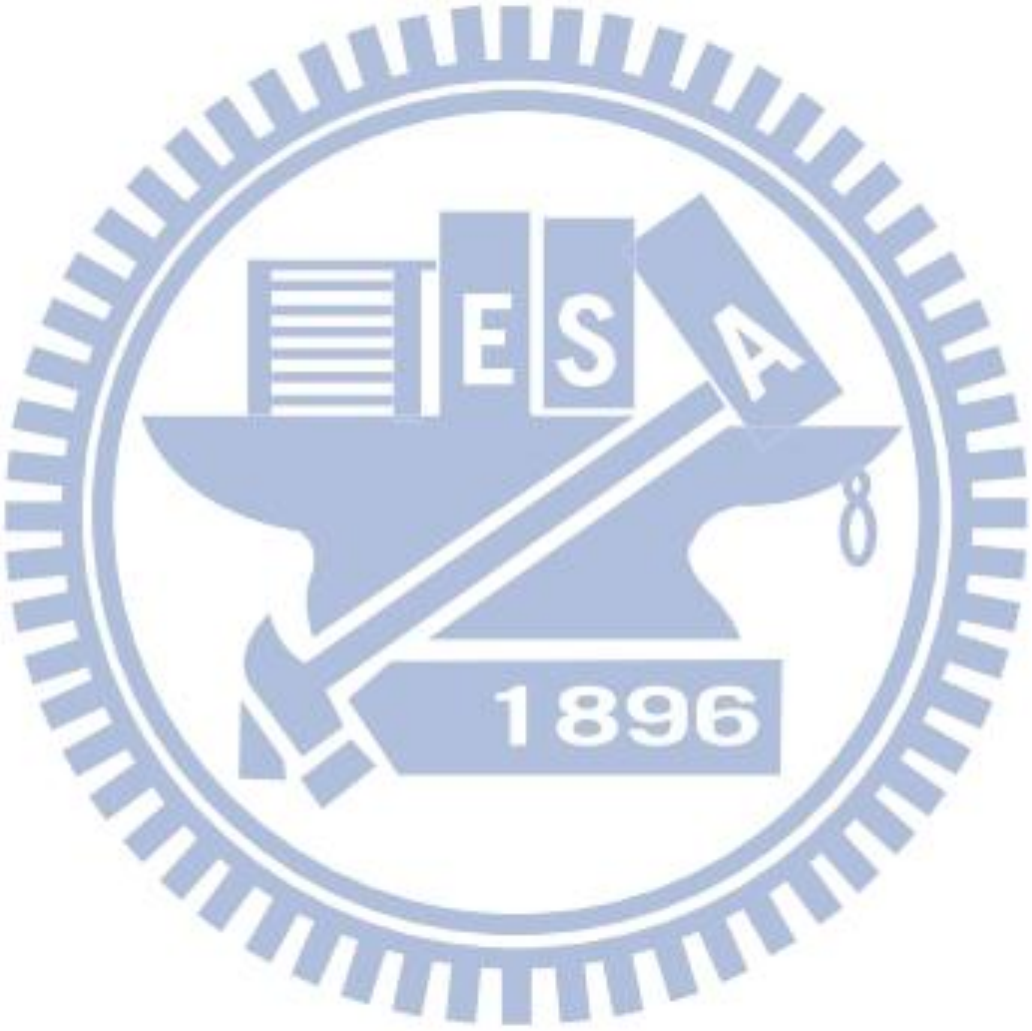
1.10.5 Microalgae in wastewater treatment

Wastewater rich in CO_2 provides a beneficial growth medium for microalgae because the CO_2 balances the Redfield ratio (molecular ratio of carbon, nitrogen and phosphorus in marine organic matter, C:N:P = 106:16:1) of the wastewater allowing for faster production rates, reduced nutrient levels in the treated wastewater, decreased harvesting costs and increased lipid production (Lundquist, 2008).

Several applications in wastewater treatment have been reported. For example, Sawayama et al. (1995) used *B. braunii* to remove nitrate and phosphate from sewage after primary treatment along with the production of hydrocarbon-rich biomass. Martínez et al. (2000) achieved a significant removal of phosphorus and nitrogen from urban wastewater using the microalgal *S. obliquus*. They were able to achieve 98% elimination of phosphorus and a complete removal (100%) of ammonium in a stirred culture at 25℃ over 94 and 183 h retention time, respectively. Gomez Villa et al. (2005) experimented with outdoor cultivation of microalgal *S. obliquus* in artificial wastewater, and achieved final dissolved nitrogen concentrations which were 53% and 21% of initial values in winter and summer, respectively. Yun et al. (1997) successfully grew *C. vulgaris* in wastewater discharge from a steel plant to achieve an ammonia (NH_3) bioremediation rate of 0.022 g L^{-1} NH_3 per day. To improve efficiencies, Muñoz et al. (2009) found the use of a biofilm attached onto the reactor walls of flat plate and tubular photobioreactors improved BOD_5 removal rates by 19% and 40%, respectively, when compared with a control suspended bioreactor for industrial wastewater effluent. The retention of algal biomass showed remarkable potential in maintaining optimum microbial activity while remediating the effluent.

For processing of hazardous or toxic compounds, it is possible to use microalgae to generate the oxygen required by bacteria to biodegrade pollutants such as polycyclic aromatic hydrocarbons (PAHs), phenolics and organic solvents. Photosynthetic oxygen from microalgae production reduces or eliminates the need for external mechanical aeration (Muñoz and Guieysse, 2006). Chojnacka et al. (2004) found that *Spirulina* sp. acted as a biosorbent, thus was able to absorb heavy metal ions (Cr^{3+}, Cd^{2+} and Cu^{2+}). Biosorption properties of microalgae depended strongly on cultivation conditions with photoautrophic

species showing greater biosorption characteristics.



2. Research Approaches

Global warming induced by increasing concentrations of greenhouse gases in the atmosphere is of great concern. CO_2 is one of the main greenhouse gases emitted to the atmosphere. And now the drastic energy crisis is due to the shortage of oil and additionally to electricity or other natural resources. Biological method, particularly using microalgal photosynthesis, is the most promising potential method for CO_2 sequestration from flue gas. In addition, microalgae convert CO_2 from a point source into biomass and use CO_2 efficiently and accumulate into lipid as a biodiesel source. Therefore, CO_2 removal and utilization by the use of microalga is a potential method for directly removing CO_2 and biodiesel production. Flue gases from power plant are responsible for more than 7% of the world CO_2 emissions from energy use and steel plants are the single largest source of energy-related CO_2 emissions in the world. In general, the primary emission in 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. This CO_2 is a plentiful carbon source for microalgal cultures. The direct use of the flue gas reduces the cost of pretreatment. However, the direct use of the flue gas 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. Temperature is also an inhibitory growth factor for outdoor microalgal cultivation.

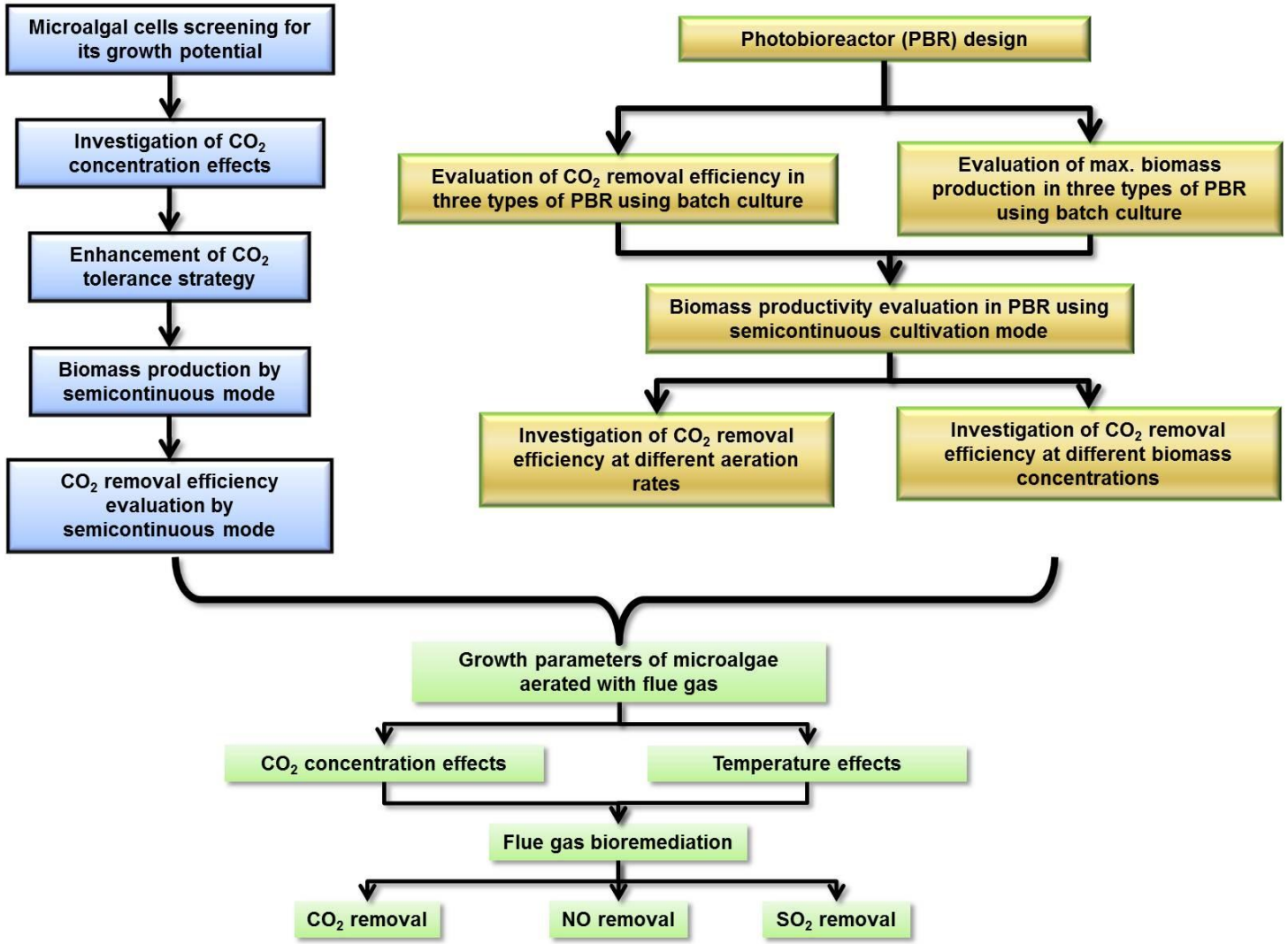
Even though that the microalgae have the widen areas of characterization, the microalgae have to be improved to select more potential strains which show the potential of biomass production and remove more CO_2. Numerous studies have been carried out to screen and isolate the potential microalgal strains. For examples, isolation of high lipid content microalgae that could be cultivated in open ponds using CO_2 from coal fired power plants for wide-scale renewable fuel (biodiesel) production, isolation of *Chlorella* mutant that produces more chlorophyll and carotenoids than a wild-type strain for heterotrophic cultures, isolation of starchless mutant strain of *Chlorella pyrenoidosa*, displays a significantly higher growth rate in both batch and continuous cultivation.

For on-site bioremediation of CO_2, NO_X and SO_X from flue gas, first, we aimed to characterize the effect of CO_2 on biomass production. In addition, we also established photobioreactor system which microalgae can grow rapidly and can be readily incorporated into the engineered systems. There are lots of researches and applications on the large scale outdoor photobioreactor cultivation system for the potential in CO_2 reduction. However, there

are limited literatures reported about on-site bioremediation by microalgal cultures and the performance of outdoor enclosed photobioreactors still can not even achieve the values obtained at laboratory scale.

In the present study, we isolated thermal- and flue gas tolerant mutant strains, *Chlorella* sp MTF-7, which can grow well in an outdoor closed photobioreactor for bioremediation under strong sunlight without the supply of cooling system in the area of subtropical region. The growth potential, operation strategy of outdoor photobioreactor system and the performance of on-site bioremediation of CO_2, NO_X and SO_X from flue gas were investigated. The whole research flow chart is as follows:

Flow chart of microalgal biomass production and bioremediation research design



3. Experimental Methodologies

3.1. Microalgal cultures, medium and chemicals

A culture of *Chlorella* sp. was obtained from Taiwan Fisheries Research Institute (Tung-Kang, Taiwan). The species of *Chlorella* sp. isolated in Taiwan was unidentified. However, the partial sequence of 18S rRNA (599 bp) of the *Chlorella* sp. has been amplified and sequenced for species identification in this study. The result of sequence alignment was performed by NCBI nucleotide blast (Wu et al., 2001). The result indicates that the *Chlorella* sp. is identified as several *Chlorella* sp. strain, such as KAS001, KAS005, KAS007, KAS012, MBIC10088, MDL5-18 and SAG 211-18. The microalga *N. oculata* NCTU-3 was originally obtained from the collection of Taiwan Fisheries Research Institute (Tung-Kang, Taiwan) and screened for its potential ability of growth and biomass production at National Chiao Tung University, Taiwan (data not shown). The cells of *Chlorella* sp. and *N. oculata* were cultured in the modified f/2 medium in artificial sea water (per liter), including 29.23 g NaCl, 1.105 g KCl, 11.09 g $MgSO_4 \cdot 7H_2O$, 1.21 g Tris-base, 1.83 g $CaCl_2 \cdot 2H_2O$, 0.25 g $NaHCO_3$, and 3.0 mL of trace metal solution (Guillard, 1975). The trace metal solution (per liter) contains 281.3 mg $NaNO_3$, 21.2 mg $NaH_2PO_4 \cdot H_2O$, 16.35 mg $Na_2 \cdot EDTA$, 11.8 mg $FeCl_3 \cdot 6H_2O$, 675 μg $MnCl_2 \cdot 4H_2O$, 37.5 μg $CoCl_2 \cdot 6H_2O$, 37.5 μg $CuSO_4 \cdot 5H_2O$, 82.5 μg $ZnSO_4 \cdot 7H_2O$, 22.5 μg Na_2MoO_4, 0.375 mg vitamin B_1, 0.188 μg vitamin B_{12} and 0.188 μg biotin.

3.2. Experimental system with photobioreactor

The microalga was incubated in a cylindrical glass reactor (30 cm length, 7 cm diameter) with 800 mL of working volume. The photobioreactor for microalgal culture and CO_2 reduction is presented schematically in **Figure 2**. Cultures were placed on a bench at 26 ± 1℃ under continuous, cool white, fluorescent light. Light intensity was approximately 300 μmol m^{-2} s^{-1} at the surface of the photobioreactor. Filtered (0.22 μm) ambient air was mixed with CO_2 to give concentrations of CO_2 of 2%, 5%, 10%, and 15%. Cultures in the photobioreactor were aerated continuously with one of the mixtures at a rate of 200 mL min^{-1} (i.e., 0.25 vvm, volume gas per volume media per min).

3.3. Preparation of the inoculum

A stock culture of microalgal cells (approximately 1×10^5 cells mL^{-1}) was incubated in an Erlenmeyer flask containing 800 mL working volume of modified f/2 medium at 26 ± 1℃ and 300 μmol m^{-2} s^{-1}. Six days after inoculation, microalgal cells were harvested by centrifugation at 3,000 × *g* for 5 min, after which the pelleted cells were resuspended in 50 mL modified f/2 medium. The density of cells in the culture was then measured and the cells were separated for the further experiments.

3.4. Experimental design of indoor batch cultivation

The photobioreactor was filled with 750 mL modified f/2 medium. The medium was aerated for 24 hours and then inoculated with 50 mL of precultured *Chlorella* sp. containing either 8×10^5 cells mL^{-1} (low-density) or 8×10^6 cells mL^{-1} (high-density). The cells from a 50 mL (at the density of 3.2×10^7 cells mL^{-1}) of precultured micralgal *Chlorella* sp. were subcultured into the 800 mL culture photobioreactor as low-density and the tenfold concentrated micralgae by centrifugation were subcultured into the photobioreactor as high-density culture. Air of different CO_2 concentration was produced by mixing air and pure CO_2 at 0.25 vvm. Each air/CO_2 mixture was adjusted to desired concentration of 2%, 5%, 10%, and 15% CO_2 in airstreams. Cultures were incubated for 4-8 days. Every 8 hours, each culture was sampled to determine optical density, microalgal dry weight, and culture pH.

3.5. Experimental design of indoor semicontinuous cultivation

The semicontinuous cultivation system was setup in a single photobioreactor and a system with six-parallel photobioreactor. Each unit of photobioreactor contained 800 mL cultured microalgae. The culture was started as a batch culture. Precultured microalgae were inoculated into the photobioreactor under 2% CO_2 aeration. When cell density reached about 1×10^8 cells mL^{-1} (the value of $A_{682} > 5$), half of volume of the culture broth was replaced with fresh modified f/2 medium every 24 hours. In each photobioreactor, the culture was aerated with 2%, 5%, 10% and 15% CO_2 at 0.25 vvm. Before fresh medium was added, the culture broth was sampled to estimate optical density, microalgal dry weight, lipid content, and pH. The amount of CO_2 reduced from the airstreams was estimated from the difference between the CO_2 concentrations in influent and effluent airstreams of the photobioreactors.

For the maintenance of biomass production, a semicontinuous culture mode was applied.

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

3.6. Microalgal cell counting and dry weight

A direct microscopic count was performed on the sample of microalgal suspension using a Brightline Hemacytometer (BOECO, Hamburg, Germany) and a Nikon Eclipse TS100 inverted metallurgical microscope (Nikon Corporation, Tokyo, Japan). Cell density (cells mL^{-1}) was measured by the absorbance at 682 nm (A_{682}) in an Ultrospec 3300 pro UV/Visible spectrophotometer (Amersham Biosciences, Cambridge, UK). Each sample was diluted to give an absorbance in the range of 0.1–1.0 due to the biomass will be underestimated when the optical density is out of the linear range. Therefore, the sample was diluted to measure getting an absorbance in the range 0.1–1.0. Microalgal dry weight (g L^{-1}) was measured according to the method previously reported (American Public Health Association, 1998). Culture broth of samples was removed by centrifugation and washed twice with deionized water. Finally, the microalgal pellet was collected from the deionized water by centrifugation. Dry weight was measured after drying the microalgal pellet at 105℃ for 16 hours (Takagi et al., 2006).

3.7. Measurement of growth rate

The optical density of microalgal cells was converted into dry weight per liter of culture by a regression equation. Biomass was calculated from microalgal dry weight produced per liter (g L^{-1}). Specific growth rate (μ, d^{-1}) was calculated from

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

where W_f and W_0 were the final and initial biomass concentration, respectively. $\triangle t$ was the

cultivation time in day (Ono and Cuello, 2007). Thus, we used biomass (g L^{-1}) to quantify microalgal cells in culture.

3.8. pH and light measurements

Sample pH was directly determined using an ISFET pH meter KS723 (Shindengen Electric Mfg.Co.Ltd, Tokyo, Japan). The pH meter was calibrated daily using pH 4 and 7 solutions. Light intensity was measured adjacent to the bioreactor at liquid level using a Basic Quantum Meter (Spectrum Technologies, Inc., Plainfield, IL).

3.9. Lipid extraction and measurement

For the lipid extraction, the microalgal cells were obtained by centrifuging a 50-mL sample of culture at 3,000 × *g* for 15 min. The cells were washed with deionized water twice, lyophilized, and weighed. A sample (30 mg) was precipitated in methanol/chloroform (2/1, v/v) and sonicated for 1 hour. Chloroform and 1% NaCl were added to give mixture to a ratio of methanol, chloroform, and water of 2:2:1. The mixture was centrifuged at 1,000 × *g* for 10 min and the chloroform phase was collected. Chloroform was evaporated under vacuum in a rotary evaporator to remove organic solvent. The remaining from the evaporation was weighed as lipid (Takagi et al., 2006).

3.10 Measurement of lipid content by fluorescent spectrometry

For fast determination of lipid content, a fluorescent spectrometric method was applied. In the method, the microalgal cells were stained with Nile Red (Sigma, St. Louis, MO, USA) followed the protocol reported by de la Jara et al. (2003). In brief, 1 mL of 1×10^6 cells suspension was added 50 μL of Nile Red in acetone working solution as a concentration of 0.1 mg mL^{-1} for lipid staining. The mixture was gently inverted for mixing and incubated at 37℃ in darkness for 10 min. In the detection, the fluorometer with a 480 nm excitation filter and a 580 nm emission filter was used. Non-stained cells were used as an auto-florescence control. The relative florescence intensity of Nile Red was calculated as florescence intensity of Nile Red stained subtracted auto-florescence intensity signal (Lee et al., 1998; Liu et al., 2008). The following equation of the correlation curve indicated fluorescent intensity of Nile Red staining *vs.* lipid content measured by gravimetric method.

$y = 1.680x + 5.827$ $R^2 = 0.994$ ($p < 0.001$)

The value y is total lipid content determined by gravimetric method. The value x is the relative arbitrary unit obtained Nile Red fluorescent spectrometric method.

3.11 Measurement of medium nitrate content

Depletion of nutrients in microalgal culture was monitored by the determination of medium nitrate content (Tonon et al., 2002). It is a simple method for investigating the adequate content of nutrients for microalgal culture in the semicontinuous cultivation. Nitrate concentration was determined according to the method reported by Collos et al. (1999). A sample collected from photobioreactor was centrifuged at 3,000 × *g* for 5 min. The supernatant was collected and the absorbance was measured at 220 nm. Authentic sodium nitrate at a concentration of 0 to 440 μM was used as a standard.

3.12 Determinations of $CO_{2(g)}$ and $CO_{2(aq)}$

The CO_2 concentration in airstreams, $CO_{2(g)}$, was measured using a Guardian Plus Infra-Red CO_2 Monitor D-500 (Edinburgh Instruments Ltd, Livingston, UK). Free CO_2 in the aqueous solution, $CO_{2(aq)}$, was measured by a HANNA Carbon Dioxide Test Kit (KI 3818; Hanna Instruments, Woonsocket, RL).

3.13 Photobioreactors and operation of microalgal culture

Three types of photobioreactor were designed and used in this study: (i) without inner column (i.e. a bubble column), (ii) with a centric-tube column and (iii) with a porous centric-tube column (**Figure 3**). The working volume in the photobioreactors was 4 L. The gas supply is from the bottom of the photobioreactor. The photobioreactors were placed in an incubator at 26±1℃, with a light intensity of approximately 300 μmol m^{-2} s^{-1} at the surface of the photobioreactor provided by a continuous, cool white, fluorescent light source. The outer column with a diameter of 100 mm was made of glass, and the inner column with a diameter of 45 mm was made of acrylics. The heights of the outer glass column and the inner acrylic column were 650 and 600 mm, respectively. The dimensions of the three photobioreactors are also shown in **Figure 3**. The flow pattern was determined through the dye technique. Batch

cultures were used to inoculate the three types of photobioreactor (without inner column, with centric-tube column, and with porous centric-tube column) at an initial biomass concentration of 1.0 g L^{-1}, and the cultures were aerated with 5% CO_2 at an aeration rate of 1.0 L/min (i.e. 0.25 vvm).

3.14 Determination of CO_2 removal efficiency

The CO_2 concentration in the airstreams was sampled as influent and effluent load and measured using a Guardian Plus Infra-Red CO_2 Monitor D-500 (Edinburgh Instruments, Livingston, UK). The efficiency of CO_2 removal was determined by the difference of the CO_2 concentration between the influent and the effluent load of the photobioreactor with the microalgal culture. The removal efficiency (%) was determined by the following formula:

$$\frac{\text{Influent of } CO_2 - \text{ Effluent of } CO_2}{\text{Influent of } CO_2} \times 100\%$$

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

3.15. Experimental system of indoor photobioreactor for on-site bioremediation experiments

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℃ 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 CO_2-enriched gas was premixed with air and pure CO_2 for the flue gas experiments as a control gas. In the gas airstream, CO_2 concentration was adjusted to 2, 10 and 25% for cultures as control experiments. The flue gas (approximately 25% CO_2, 4% O_2, 80 ppm NO and 90 ppm SO_2) 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.

3.16.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 CO_2, , O_2, NO and SO_2 in the flue gas were 23 ± 5%, 4.2 ± 0.5%, 78 ± 4 ppm and 87 ± 9 ppm (Oct. 1 – Nov. 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 CO_2, O_2, NO and SO_2.

3.17. Chemical analyses

In the on-site bioremediation experiments, the inlet and outlet loads of airstreams were real-time monitored by a gas analyzer. The concentration of O_2, CO_2, NO and SO_2 in flue gas were measured using Landcom III portable gas analyzer (AMETEK, Inc., Paoli, PA, USA).

3.18 Statistics

All values are expressed as mean ± standard deviation (SD). A Student's *t* test was used to evaluate differences between groups of discrete variables. A value of $P < 0.05$ was considered statistically significant.

4. Part I: Reduction of CO_2 by a high-density culture of *Chlorella* sp. in a semicontinuous photobioreactor

4.1 Introduction

Global warming induced by increasing concentrations of greenhouse gases in the atmosphere is of great concern. CO_2 is the principal greenhouse gas and its concentrations have increased rapidly since the onset of industrialization (Ramanathan, 1988). In 1997, 7.4 billion tons of CO_2 were released into the atmosphere from anthropogenic sources; by the year 2100, this number will increase to 26 billion tons (DOE, 1999). During the last two decades, many attempts have been made to reduce atmospheric CO_2, for example by the use of renewable energy sources or by terrestrial sequestration of carbon (IPCC, 2001).

One of the most understudied methods of CO_2 reduction is the use of microalgae that convert CO_2 from a point source into biomass. Microalgae use CO_2 efficiently because they can grow rapidly and can be readily incorporated into engineered systems, such as photobioreactors (Carvalho et al., 2006; Lee and Lee, 2003; Suh and Lee, 2003).

The CO_2 fixation rate is related directly to light utilization efficiency and to cell density of microalgae. Microalgal CO_2 fixation involves photoautotrophic growth in which anthropogenically derived CO_2 may be used as a carbon source. Therefore, biomass measurements or growth rate evaluations are critical in assessing the potential of a microalgal culture system for directly removing CO_2 (Chen et al., 2006; Costa et al., 2004; Jin et al., 2006). Effects of the concentration of CO_2 in airstreams on growth of microalgae in culture have been evaluated in several studies (Chae et al., 2006; de Morais and Costa, 2007; Keffer and Kleinheinz, 2002; Kurano et al., 1995; Yoshihara et al., 1996). However, these effects remain to be largely understood. Microalgal photobioreactor can be used for CO_2 mitigation from waste gas with high concentration of CO_2 efficiently, if the effects of the CO_2 concentration in airstreams on microalgal cell growth could be well controlled.

In the present study, effects of initial cell density and CO_2 concentration in airstreams on growth of a *Chlorella* sp. culture were investigated. The efficiency of CO_2 reduction and biomass as well as lipid productivity in a semicontinuous photobioreactor system were also evaluated.

4.2 Results and discussion

4.2.1. Evaluation of cell density and biomass

Cell density and biomass were measured more easily by optical density than by direct counting of cells or by cell dry weight (Rocha et al., 2003). Therefore, relationships between optical density and cell density and optical density and cell dry weight were established by linear regression firstly (**Figure 4**). Optical density precisely predicted both cell density (R^2 = 0.997; $p < 0.001$) and biomass (R^2 = 0.991; $p < 0.001$). Therefore, the values of optical density were used to calculate the related biomass of *Chlorella* sp. in each experiment according the equations established.

4.2.2. Effect of CO_2 on microalgal culture at different cell density

To investigate the effect of CO_2 concentration on growth, *Chlorella* sp. in batch culture was incubated for 4 to 8 days at 26 ± 1℃ and 300 μmol m^{-2} s^{-1} and aerated with different concentrations of CO_2 at 0.25 vvm. Cultures were sampled when a stationary phase of growth was reached or a microalgal growth was significantly inhibited. Specific growth rate was calculated from the logarithmic growth phase over 1 to 2 days batch culture in each experiment.

As the cells grew up to plateau stage, the biomass in air, 2% and 5% CO_2 aeration with low-density cells inoculum (i.e., 8×10^5 cells mL^{-1}) were 0.537 ± 0.016 g L^{-1}, 1.211 ± 0.031 g L^{-1} and 0.062 ± 0.027 g L^{-1}, respectively. At the aeration of 2% CO_2, *Chlorella* sp. increased most rapidly at the specific growth rate of 0.492 d^{-1} and the specific growth rate markedly fell to be 0.127 d^{-1} when the cultures were aerated with 5% CO_2. The growth of *Chlorella* sp. at 10% and 15% CO_2 aeration was almost completely inhibited; therefore the specific growth rates were not available (**Figure 5A** and **Table 5**).

In the cultures inoculated with *Chlorella* sp. at high-density (i.e., 8×10^6 cells mL^{-1}), a short lag period and steep log phase was observed when the cultures aerated with 2% and 5% CO_2 compared to those of low-density inoculum. It is worth to emphasize that the biomass and specific rate at 5% CO_2 aeration in high-density inoculum was 0.899 ± 0.003 g L^{-1} and 0.343 d^{-1}. The values were significantly increased as compared with those in low-density inoculum. However, the growth of *Chlorella* sp. was inhibited after 4 days of incubation under the conditions of 10% and 15% CO_2 aeration (**Figure 5B** and **Table 5**). In the 5% CO_2

aerated cultures in high-density inoculum, the biomass production and specific growth rate were strongly enhanced. This enhancement may due to enrichment of available CO_2 as carbon source and the culture condition under the 5% CO_2 aeration would not be significantly changed in the culture with higher cell density inoculated.

Chlorella sp. grew rapidly in a high-density culture with CO_2 aeration. The result is confirmed by the report that the waste gas or CO_2 tolerance of microalgae was dependent on cell density (Lee et al., 2002; Yoshihara et al., 1996; Yun et al., 1997).

4.2.3. Effect of CO_2 on cell growth in semicontinuous cultivation

The semicontinuous culture was carried out in two stages. A batch culture had an initial cell density of 8×10^6 cells mL^{-1} (i.e., a high-density of inoculum). At 2% CO_2, cell density was allowed to increase until it reached an optical density (A_{682}) over 5 (the cell density was around 1×10^8 cells mL^{-1}), which occurred after 6 to 8 days of incubation. After that, half of the culture broth was replaced with fresh modified f/2 medium each day and the culture was incubated with 2%, 5%, 10%, and 15% CO_2 aeration. The growth of *Chlorella* sp. in the semicontinuous culture was constantly similar at 2%, 5%, 10%, and 15% CO_2. The average specific growth rate and biomass, respectively, were 0.58 to 0.66 d^{-1} and 0.76 to 0.87 g L^{-1} after 8 days of incubation at 2 to 15% CO_2 aeration. These semicontinuous cultures aerated with different CO_2 concentrations were operated for 24 days. The growth of these cultures was stable on each day. These results shows that a high concentration of CO_2 (10-15%) may directly introduce to a high-density *Chlorella* sp. culture in the semicontinuous photobioreactor system. The high CO_2 concentration did not cause harmful effects on microalgae, indicating that the CO_2 can be as carbon source for the growth of a variety of photosynthetic microalgae at high-density culture. An initial high-density of the *Chlorella* sp. cultures that was adapted to 2% CO_2 may overcome environment stress induced by higher CO_2 (10-15%) aeration.

Selection of the mutant of *Chlorella* sp. represents one approach to elevating CO_2 tolerance of microalgae (Chang and Yang, 2003). However, growth and cell density in the cultures aerated with high levels of CO_2 are still limited in the application of these mutants. Chang and Yang (2003) have isolated *Chlorella* strains NTU-H15 and NTU-H25 and found that the greatest biomass produced by each strain at 5% CO_2 was 0.28 g L^{-1} d^{-1}. The other mutant, *Chlorella* strain KR-1, showed a potential biomass of 1.1 g L^{-1} d^{-1} at 10% CO_2 (Sung

et al., 1999). However, increasing the cell density in the cultures or pre-adapting cells in a low concentration of CO_2 are alternative approaches to increase CO_2 tolerance of microalgae without effects on microalgal growth (Lee et al., 2002; Yun et al., 1997). In our semicontinuous photobioreactors, *Chlorella* sp. cells that were pre-adapted to 2% CO_2 not only grew into a high-density microalgal culture but also grew fast at 10% or 15% CO_2. Our results confirmed these previous studies and provided a useful system that can be applied to conversion of CO_2 into biomass.

4.2.4. Effect of CO_2 on CO_2 reduction in semicontinuous culture

Semicontinuous *Chlorella* sp. culture was conducted to examine the potential of CO_2 reduction in the photobioreactor using a high-density culture. Prior to the photobioreactor being operated with microalgae present, the photobioreactor was emptied and operated for 1 day without microalgae to test for any abiotic removal of CO_2, at 2%, 5%, 10%, and 15% CO_2. During these tests, the average influent and effluent concentrations of CO_2 were similar. Thus, CO_2 was not removed via an abiotic mechanism.

The amount of CO_2 reduced from the airstreams was estimated in the semicontinuous *Chlorella* sp. cultures during an 8-day period. The difference in CO_2 concentration between the influent load and effluent load were monitored. All runs in each treatment and on each day were remarkably consistent and showed a similar pattern among the influent and effluent CO_2 measurements. The effluent CO_2 concentrations in the influent 2%, 5%, 10% and 15% CO_2 treatments was maintained at 0.8~1.0%, 3.5~3.8%, 7.9~8.4% and 12.4~12.8% CO_2 during 8-day operation, respectively. The average rate of CO_2 reduction in cultures at 2%, 5%, 10%, and 15% CO_2 in the single photobioreactor was 0.261, 0.316, 0.466 and 0.573 g h^{-1}, respectively. Thus, the overall efficiency of CO_2 reduction in the cultures was 58%, 27%, 20% and 16%, respectively (**Figure 6**). Recently, de Morais and Costa (2007a) reported greater efficiency of CO_2 fixation in cultures at low CO_2 concentration (6%) than in cultures at high CO_2 concentration (12%). The increasing retention of CO_2 in a microalgal photobioreactor also could significantly enhance the efficiency of CO_2 fixation (Cheng et al., 2006). Keffer and Kleinheinz (2002) demonstrated that air dispersed in photobioreactors operated under approximately 2 seconds of air retention time removed up to 74% of CO_2 from an airstreams containing 0.16% CO_2. The air retention time was around 1–1.5 second in our photobioreactor; therefore, we believe that amount and efficiency of CO_2 reduction can be improved by increasing the CO_2 retention time in the photobioreactor system.

In the absence of microalgae, the medium pH was 7.5 in cultures aerated with air and pH dropped to about 6.4, 6.1, 5.8, and 5.6 at 2%, 5%, 10% and 15% CO_2, respectively. However, pH was greater in each culture of inoculated with *Chlorella* sp. Average pH was 7.6, 7.4, 7.1 and 6.8 at 2%, 5%, 10% and 15% CO_2, respectively. Free CO_2 in culture broth containing *Chlorella* sp., i.e., $CO_{2(aq)}$, was also measured. The $CO_{2(aq)}$ in the cultures was stable throughout the period of 8 days of incubation. Average $CO_{2(aq)}$ in cultures aerated with 2%, 5%, 10% and 15% CO_2 was 575, 605, 660 and 705 ppm, respectively. These values were consistent with the changes in culture pH. The $CO_{2(aq)}$ concentration was generally increased with increased influent CO_2 concentration; however, the result indicates the limit on the amount of CO_2 that can dissolve in the culture broth. Most of the influent CO_2 flowed out of the photobioreactor directly when the CO_2 concentration was more than 2%.

The efficiency of CO_2 removal or fixation in a closed culture system is dependent on the microalgal species, CO_2 concentration, and photobioreactor (Cheng et al., 2006; de Morais and Costa, 2007a). Cheng et al. (2006) have demonstrated that CO_2 removal efficiency peak (55.3%) at 0.15% CO_2 and the amount of CO_2 reduction (about 80 mg L^{-1} h^{-1}) peaks at 1% CO_2 in a *Chlorella vulgaris* culture in a membrane photobioreactor. In a three serial tubular photobioreactor, 27 to 38% and 7 to 13% of CO_2, respectively, was fixed by *Spirulina* sp. and *Scenedesmus obliquus* in cultures aerated with 6% CO_2 aeration. In treatments of 12% CO_2 aeration, CO_2 fixation efficiency was only 7~17% for *Spirulina* sp. and 4~9% for *S. obliquus* (de Morais et al., 2007a). The species dependence of efficiency of CO_2 removal or fixation may be due to physiological conditions of microalgae, such as potential of cell growth and ability of CO_2 metabolism.

4.2.5. Effect of CO_2 on lipid and biomass production in semicontinuous culture

Lipid and biomass productivity in the semicontinuous *Chlorella* sp. cultures were determined before the culture broth was changed each day. **Table 6** summarizes the results, lipid and biomass productivity, collected from the single photobioreactor cultures under different CO_2 aeration. As a daily 50% culture broth replaced in the 800 mL semicontinuous photobioreactor aerated with 2%, 5%, 10%, and 15% CO_2, the total biomass and lipid productivity per day (400 mL of waste broth was recovered for measurement) of each photobioreactor was 0.422 g d^{-1}, 0.393 g d^{-1}, 0.366 g d^{-1} and 0.295 g d^{-1}, and 0.143 g d^{-1}, 0.130 g d^{-1}, 0.124 g d^{-1} and 0.097 g d^{-1}, respectively. In the single semicontinuous culture, both of lipid and biomass productivity decreased when the aerated CO_2 concentration was

increased. However, lipid content in the cells cultured at 2%, 5%, 10%, and 15% CO_2 were very similar (approximately 32~34% of dry weight). In the semicontinuous culture, the optimum condition for biomass productivity was at 2% CO_2 aeration and lipid content was not affected even at high CO_2 aeration. Biomass productivity at 15% CO_2 aeration was 68% of that at 2% CO_2 aeration. However, our results still show the potential growth of microalgal *Chlorella* sp. for lipid and biomass productivity in the semicontinuous system even the cells were cultivated in the condition aerated with 15% CO_2.

The lipid content of *Chlorella fusca* and *Phaeodactylum tricornutum* increased when cells were grown at increasingly higher concentrations of CO_2 (Dickson et al., 1969; Yongmanitchai and Ward, 1991). Our result was not consistent with these previous studies. Such divergent results for lipid content of microalgae cultured under CO_2 aeration may be due to differences in microalgal species, content of culture medium, and culture condition.

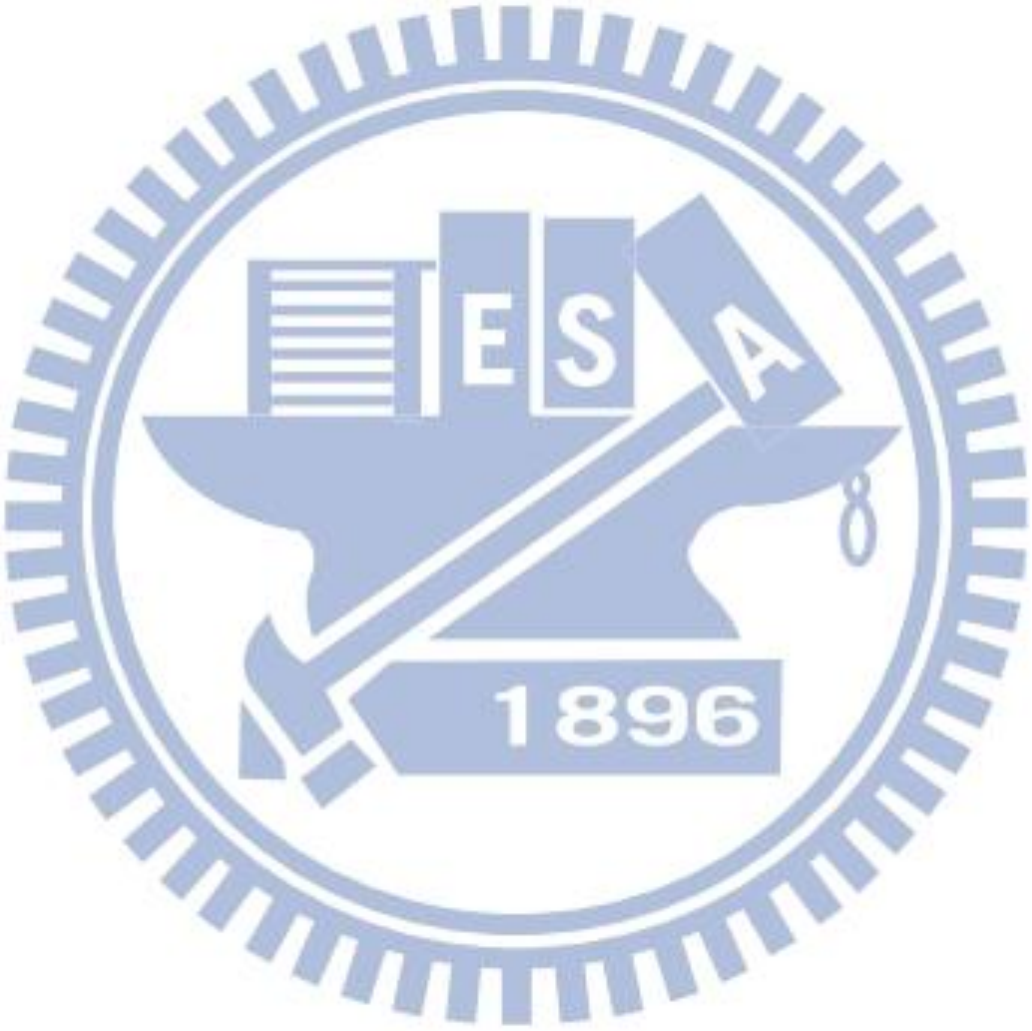
4.2.6. Performances of six-parallel photobioreactor system

The efficiency of CO_2 removal from airstreams by *Chlorella* sp. was compared between the single photobioreactor and the six-parallel photobioreactor. The effects of varying CO_2 concentration on growth of *Chlorella* sp. was similar between the single and the six-parallel photobioreactors (data not shown). In a total volume of 4,800 mL (i.e., 6 × 800 mL) of the six-parallel photobioreactor, the total amount of CO_2 reduced was 1.563, 2.058, 2.757 and 3.441 g h^{-1} at 2%, 5%, 10% and 15% CO_2 aeration, respectively (**Figure 6**). Thus, the amount of CO_2 that reduced in the six-parallel photobioreactor was approximately six times greater than the amounts in the single photobioreactor. Therefore, the efficiency CO_2 reduction in the six-parallel photobioreactor and in the single photobioreactor was also similar (**Figure 6**).

Daily recovery of lipid and biomass in the six-parallel photobioreactor were determined. In each case, the amount of lipid and biomass recovered daily in the six-parallel photobioreactor was around six times greater than the amounts recovered in the single photobioreactor (**Table 6**). CO_2 reduction efficiency and cell growth in both photobioreactor systems also were similar.

When microalgal cells grew in a closed photobioreactor, light decreases exponentially with the distance from light source (Suh and Lee, 2003). It will be a problem for diameter of scale-up photobioreactor with external lighting. Our results show that our photobioreactor could be extended to parallel multiple units of photobioreactor for discharging waste gas in a

large scale without decreasing biomass and lipid productivity, and efficiency of CO_2 reduction. Additionally, increasing the length of tubular photobioreactor and gas sparging into small bubbles can be considered in a scale-up system. Longer tubular photobioreactor and small bubbles could increase the retention time of gas in photobioreactor and the bubbles absorbed into cultures, and then increases the efficiency of CO_2 reduction.



5. Part II: Biomass production and CO_2 utilization of *Nannochloropsis oculata* in response to CO_2 aeration

5.1 Introduction

Greenhouse gases are accumulating dramatically in Earth's atmosphere as a result of human activities and industrialization. In addition, the increasing concentration of greenhouse gases causes serious global warming increasing the temperatures of the surface air and subsurface ocean. Carbon dioxide (CO_2) is the main greenhouse gas. Many attempts including physical and chemical treatments have been used to recover CO_2 from atmosphere. In biological approach, microalgae appear more photosynthetically efficient than terrestrial plants and are the candidates as efficient CO_2 fixers (Brown and Zeiler, 1993).

In recent years, the bioregenerative methods using photosynthesis by microalgal cells have been made to reduce the atmospheric CO_2 to ensure a safe and reliable living environment. As the result of mild conditions for CO_2 fixation, there is no requirement for further disposal of recovered CO_2 (Lee and Lee, 2003; Cheng et al., 2006; Jin et al., 2006). Marine microalgae are expected as a proper candidate due to their high capability for photosynthesis and easily cultured in seawater which solubilizes high amount of CO_2. The CO_2 fixation by microalgal photosynthesis and biomass conversion into liquid fuel is considered a simple and appropriate process for CO_2 circulation on Earth (Takagi et al., 2000).

Lipids from microalgae are chemically similar to common vegetable oils and have been suggested being a high potential source of biodiesel (Dunahay et al., 1996; Chisti, 2007). Microalgal oil most accumulated as triglycerides can be transformed to biodiesel (Lee et al., 1998; Zhang et al., 2003). The biodiesel compared with fossil-driven diesel, that is renewable, biodegradable, and low pollutant produced (Vicente et al., 2004). The advantages of biodiesel from microalgae are that microalgae are easy to culture and less area occupation for cultivation (Chisti, 2007). In addition, microalgal-based biodiesel is a potential renewable resource for displacement liquid transport fuels derived from petroleum (Chisti, 2008).

Nannochloropsis oculata is an interesting microorganism in the field of marine biotechnology because of its high lipid content. Many microalgae can accumulate lipids due to excess photosynthate and some species can accumulate amount of lipids under heterotroph

or environment stress, such as nutrient deficiency (Takagi et al., 2000) or salt stress (Takagi et al., 2006). In this study, we investigated the effects of CO_2 concentration in airstreams on the biomass production and lipid accumulation of *N. oculata* NCTU-3 cultures. We also evaluated the efficiency of lipid productivity in a semicontinuous system for *N. oculata* NCTU-3 cultures. For a long-term operation, the total biomass and lipid productivity in the semicontinuous system operated by one-day and three-day replacement were evaluated.

5.2 Results and Discussion

5.2.1. Growth of N. oculata NCTU-3 aerated with different CO_2 concentration

Effect of CO_2 concentration in airstream on the growth of *N. oculata* NCTU-3 was investigated in a batch culture incubated at 26 ± 1℃ and 300 μmol m^{-2} s^{-1}. The initial biomass inoculum was 0.01 g L^{-1} (about 7×10^5 cells mL^{-1}) and the cultures were aerated with air (CO_2 concentration is approximate 0.03%), 2, 5, 10, and 15% CO_2. The cultures were sampled at an 8-h interval. The specific growth rate was calculated from the cultures in each experiment. **Figure 7** shows the microalgal growth aerated with different CO_2 concentrations. After 6-8 d, the growth of air and 2% CO_2 aerated cultures were reached a plateau stage and the biomass concentration of *N. oculata* NCTU-3 were 0.268 ± 0.022 and 1.277 ± 0.043 g L^{-1}, respectively. Whereas, the growth of microalga aerated with 5, 10, and 15% CO_2 were completely inhibited. The specific growth rate in the air and 2% CO_2 aerated cultures were 0.194 d^{-1} and 0.571 d^{-1}, respectively. The culture aerated with 2% CO_2 showed an optimal growth potential. When the *N. oculata* NCTU-3 culture aerated with 2% CO_2, not only the biomass was greatly produced but also the specific growth rate was enhanced compared with those in the culture aerated with air. This result was confirmed by Hu and Gao (2003). They indicated that microalga, *Nannochloropsis* sp., grew best in an enriched CO_2 aeration compared with air aeration. It may due to enough carbon sources for microalgal growth without carbon source limitation. The significant inhibition of high CO_2 aeration, 5 to 15%, was also confirmed by the reports that the concentration of CO_2 aeration above 5% could be harmful to microalgal cells and inhibit the microalgal growth (Silva and Pirt, 1984; Sung et al., 1999; Chang and Yang, 2003; de Morais and Costa, 2007b).

5.2.2. Lipid content of microalga at different growth phases

The microalgal cells from logarithmic, early stationary phase and stationary phase were collected to measure lipid content and supernatant from the collected samples was also obtained for determining the nitrate content in broth. The result showed that the lipid accumulation in microalgal cells was associated with growth phases. The lipid content of *N. oculata* NCTU-3 cells at logarithmic, early stationary phase and stationary phase was 30.8, 39.7 and 50.4%, respectively. This result indicated that lipid accumulation increases as *N. oculata* NCTU-3 approaches into stationary phase. The decreased nitrate content in the broth of *N. oculata* NCTU-3 culture from logarithmic phase to stationary phase was found (data not shown). It is hinted that the *N. oculata* NCTU-3 culture from logarithmic phase to stationary phase would accompany with the nitrate depletion. Roessler (1988) reported that the nutrient deficiency induced an increase in the rate of lipid synthesis in a diatom, *Cyclotella cryptica*, and resulted in lipid accumulation in the cells. It is also indicated that lipid accumulation is related to nitrogen depletion as a nutrient deficiency (Roessler et al., 1994; Takagi et al., 2000). The result is confirmed by these previous reports that the microalga, *N. oculata* NCTU-3, shows the metabolic effect of nitrogen depletion related to the increasing lipid accumulation.

5.2.3. Effect of CO_2 concentration on cell growth in semicontinuous cultures

For the study of lipid accumulation in response to higher CO_2 aeration, the microalgal cells pre-adapted to CO_2 were applied. In the experiment, *N. oculata* NCTU-3 cells were pre-adapted to 2% CO_2 before the microalga was inoculated into the semicontinuous cultures. Moreover, a high density (approximate 0.4 g L^{-1}) of inoculum was applied in the cultures. The semicontinuous system was operated for 8 d and the growth was stable by each day replacement and was maintained at logarithmic growth potential. The results showed that the growth profiles of *N. oculata* NCTU-3 aerated with 2, 5, 10, and 15% CO_2 in the semicontinuous system were similar (**Figure 8**). The average specific growth rate and maximum cell density (i.e., biomass concentration) were from 0.683 to 0.733 d^{-1} and from 0.745 to 0.928 g L^{-1} at different concentrations of CO_2 aerated cultures, respectively **(Figure 8**). High CO_2 aeration (5-15%) may be a harmful effect on the microalgal cells growth as shown in Fig. 1. But increasing the inoculated cell density and pre-adapting to 2% CO_2 culture could promote the growth capacity of microalga in the cultures aerated with higher CO_2 concentrations. The results indicated that increasing cell density and pre-adapting microalgal cells in an adequate CO_2 concentration is an alternative approach for the

application of high CO_2 aeration without drastic harmful effects on microalgal cell growth.

5.2.4. Biomass and lipid productivity in semicontinuous culture

In the semicontinuous culture system, the *N. oculata* NCTU-3 cells were collected at the time before culture replaced each day for determination of biomass and lipid productivity. **Table 5** summarizes the biomass and lipid productivity of *N. oculata* NCTU-3 cultures aerated with various CO_2 concentrations. As increasing CO_2 concentration of aeration from 2 to 15%, both biomass and lipid productivity were generally decreasing (**Table 7**). It is reported that the lipid content was increasing associated with the increasing CO_2 concentration of aeration in *Chlorella fusca* and *Phaeodactylum tricornutum* cultures (Dickson et al., 1969; Yongmanitchai and Ward, 1991). The data in this study showed an inverted result may due to different microalgae species, growth condition, and medium content(Hu and Gao, 2006). Our results show that the pH of cultures with 2, 5, 10 and 15% CO_2 aeration was maintained at pH 7.8, 7.7, 7.3 and 7.0, respectively. Yung and Mudd (1966) reported that the carbon assimilation of lipid synthesis was decreased with decrease of pH. This may be possibly because the higher pH having higher available bicarbonate for carboxylation of lipid synthesis. This inference supports the result that lipid accumulated in *N. oculata* NCTU-3 may be mainly affected by pH and lipid content of the microalgal cultures was decreased with decrease of broth pH.

5.2.5. Optimal CO_2 concentration applied in semicontinuous cultures

In the semicontinuous system, *N. oculata* NCTU-3 could grow well under high CO_2 (up to 15% CO_2) aeration, shows the potential of the microalgal culture for CO_2 removal. Therefore, the CO_2 removal efficiency in the semicontinuous system cultured with *N. oculata* NCTU-3 was determined by the measurement of influent and effluent of CO_2 airstream. The method and operation was established and described in our previous study (Chiu et al., 2008).

The amount of CO_2 between influent and effluent, and CO_2 removal efficiency were recorded. The CO_2 concentrations in the effluent of 2, 5, 10 and 15% CO_2 aerated cultures were maintained at 0.9–1.1, 3.8–4.1, 8.3–8.7 and 12.9–13.2% CO_2 over 8 d cultivation, respectively. The CO_2 removal efficiency in the cultures aerated with 2, 5, 10, and 15% CO_2 were 47, 20, 15 and 11%, and the amount of CO_2 removal in the cultures were 0.211, 0.234, 0.350 and 0.393 g h^{-1}, respectively. The efficiency of CO_2 removal in the cultured aerated

with low CO_2 concentration was higher than those aerated with high CO_2 concentration (de Morais and Costa, 2007a; Chiu et al., 2008). The CO_2 removal efficiency in a closed photobioreactor system is dependent on microalgal species, photobioreactor, and concentration of CO_2 aeration (Cheng et al., 2006; de Morais and Costa, 2007a). This assumption was confirmed by the study in *Chlorella* sp., the study showed more CO_2 removal capacity but lower biomass productivity in a microalgal culture treated with low CO_2 aeration (Chiu et al., 2008). Cheng et al. (2006) demonstrated a *Chlorella vulgaris* cultured membrane-photobioreactor obtained a maximum rate of microalgal CO_2 fixation at 2% of CO_2 aeration. Different photobioreactors could also bring different gaseous transfer efficiency, light harvesting efficiency, and mix efficiency (Carvalho et al., 2006). In the present study, amount of CO_2 removal was 0.264, 0.293, 0.438 and 0.491 g L^{-1} h^{-1}, however, total biomass productivity was 0.480, 0.441, 0.398 and 0.372 g L^{-1} d^{-1} in the cultures with 2, 5, 10 and 15% CO_2 aeration, respectively. The microalgal cultures aerated with higher CO_2 showed lower biomass productivity. This result may due to that when the microalgal cells aerated with higher CO_2, most of the CO_2 is consumed for metabolic activity and less of CO_2 is fixed to become cellular components, i.e., biomass. The higher metabolic activity may contribute to the microalgal cells to subsist on higher CO_2 stress. The results showed that the maximal CO_2 utilization efficiency was from the cultures aerated with 2% CO_2 airstreams. It is also indicated that the optimal concentration of CO_2 aeration in the system based on the efficiency of biomass and lipid productivity was 2% CO_2.

5.2.6. Comparison of productive efficiencies in semicontinuous system with different culture approaches

The comparison of productive efficiencies in the semicontinuous systems in which the culture broth were replaced at an interval of 24 h (one-day replacement) or 72 h (three-day replacement) was performed. In the systems, approximate 0.4 g L^{-1} of *N. oculata* NCTU-3 cells was inoculated and the microalgal cultures were replaced half (for one-day replacement) or three fifth (for three-day replacement) of broth with fresh medium in the semicontinuous system after the cultures aerated with 2% CO_2. **Figure 9** shows the stable growth profiles of *N. oculata* NCTU-3 cultured with one-day and three-day replacement. In the cultures, the broth was replaced at logarithmic phase in one-day replacement and replaced before the cells reached to early stationary phase in three-day replacement. The growth profiles of both one-day and three-day replacement cultures were stable over 12 d cultivation. **Table 8** shows

the biomass and lipid productivity of *N. oculata* NCTU-3 cells in the semicontinuous culture system with one-day and three-day replacement. The total volume of replaced broth was 4,800 mL in one-day replacement and only 1,920 mL in three-day replacement over 12 d. The lipid content of microalga in the three-day replacement was significantly higher than that in the one-day replacement culture (41% *vs.* 31%). However, the total biomass and total lipid yield in the three-day replacement culture were only 24% and 32% compared with those in the one-day replacement culture, respectively. It means that the culture broth being daily replacement could be more efficient not only for biomass production but also for lipid yield. In conclusion, the total biomass and lipid yield in the semicontinuous culture operated by one-day replacement were more efficient compared with those in three-day replacement, although the *N. oculata* NCTU-3 cells in the three-day replacement could increase lipid accumulation because of nutrition-deficient effect.

6. Part III: The air-lift photobioreactors with flow patterning for a high-density culture of microalgae and carbon dioxide removal

6.1 Introduction

Global warming which results from increasing concentration of CO_2 has become an important issue of environmental concerns. There are many attempts for CO_2 recovery including physical, chemical, and biological methods (Abu-Khader, 2006; Lee and Lee, 2003). Among these attempts, the biological method using microalgal photosynthesis is believable as an effective approach for biological CO_2 fixation (Yanayi et al, 1995; Wang et al., 2008). By the biological approaches, CO_2 can be fixed into microalgal biomass by photosynthesis. The photosynthetic organisms can produce proteins, fatty acids and pigments as dietary supplements for humans and animals (Ono and Cuello, 2004). Furthermore, lipids from microalgae are chemically similar to common vegetable oils and are high potential sources for biodiesel production (Chisti, 2007). The microalgal-based biodiesel compared with fossil fuels, that is renewable, biodegradable, and low pollutant produced (Chisti, 2008; Vicente et al,, 2004). Thus, reducing atmospheric CO_2 by microalgal photosynthesis is considered safe and reliable for living environment (Lee and Lee 2003; Cheng et al., 2006).

Using outdoor microalgal cultures, such as open pond, has been proposed to reduce CO_2 emission (Jeong et al., 2003; Ono and Cuello, 2004). However, outdoor culture system is limited to microalgal growth, not easy to control the environmental parameter and shows low productivity as a result of variable environmental temperatures, system circulation and light utilization (Carvalho et al., 2006). In comparison with open culture system, closed photobioreactor is easy to control environmental parameters (Molina Grima et al., 1999) and can achieve high growth rate (Pulz, 2001; Sierra, 2008). A closed photobioreactor can be a bioscrubber for waste gas treatment and the microalgal cells cultured in the photobioreactor to convert CO_2 from the waste gas into biomass is an energy-efficient and economical approach (Chiu et al., 2008; Suh and Lee, 2003). Several studies have proved that using microalgal cells cultivated in photobioreactors is useful and practical method for CO_2 removal (Chiu et al., 2008; Ono and Cuello, 2004; Keffer and Kleinheinz, 2002). There were several types of photobioreactors, such as tubular, flat and column photobioreactors, reported. Vertical tubular type photobioreactors, such as bubble and air-lift photobioreactors, were often thought to be the most efficient mixing and the best volumetric gas transfer (Eriksen, 2008). Besides, the

traditional fermentation bioreactor, bubble column equipped with perforated draft tube has been shown significant improvements over traditional air-lift reactors in mixing and mass transfer performance (Bando et al., 1992a, b); however, the bioreactor equipped with perforated draft tube is never used in photosynthetic organism. Criteria for high-density culture in photobioreactor are good mixing, mass transfer and light utilization. In addition, in a high-density culture of microalgae, light utilization could be improved if good mixing providing the flash light effect of microalgal photosynthesis (Barbosa et al., 2002). As mentioned above, we tested whether a photobioreactor with porous centric tube is potential for the microalgal culture with high cell density.

In the present study, we designed a photobioreactor which is an air-lift type photobioreactor with porous centric tube for high-density microalgal culture and the performance evaluation was compared to the other two designs, bubble column and centric tube photobioreactors. The microalgal species, *Chlorella* sp. NCTU-2, was Taiwan native microalgal species and was screened as a potential candidate for growth and biomass production in this study. For determining the capacity of daily biomass production of the microalga, a semicontinuous culture operation was performed. Moreover, the CO_2 removal efficiency was evaluated under different aeration rates and microalgal densities (i.e., biomass concentration) of the culture.

6.2 Results and discussion

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

The comparison of the growth of *Chlorella* sp. NCTU-2 cultivated in the photobioreactors without inner column, with centric-tube column and with porous centric-tube column was performed as a batch culture in an incubator at 26±1℃, with a light intensity of approximately 300 μmol m^{-2} s^{-1} at the surface of the photobioreactor provided by a continuous, cool white, fluorescent light source. The cultures were provided with 5% CO_2 gas. The cultured samples were collected for density measurements at 12-h intervals. **Figure 10** shows the growth curves of the three cultures, and the results indicate that the microalgae cultured in the porous centric-tube photobioreactor performed at the highest growth rate.

The maximum biomass concentrations and specific growth rates of the cultures in the photobioreactors without inner column, with centric-tube column and with porous centric-tube column in batch culture mode were 2.369, 2.534 and 3.461 g L^{-1}, and 0.180,

0.226 and 0.252 d^{-1}, respectively (**Table 9**). This result indicates that the maximum biomass concentration in the porous centric-tube photobioreactor could be enhanced by 46% and 37% compared to those in the bubble column photobioreactor and in the centric-tube photobioreactor, respectively. The culture in the porous centric-tube photobioreactor showed not only an improved maximum biomass concentration but also a better specific growth rate. Recently, Ranjbar et al. (2008) reported that the maximum cell density of *Haematococcus pluvialis* cultured in an air-lift photobioreactor was 18% higher than that in a bubble column photobioreactor. Oncel and Sukan (2008) demonstrated that an air-lift photobioreactor culture yielded a maximum biomass concentration value of 2.21 g L^{-1} whereas a bubble column photobioreactor culture yielded only a maximum biomass concentration value of 1.87 g L^{-1}. The air rising randomly through the photobioreactor was the only driving force for culture mixing in the bubble column photobioreactor (Oncel and Sukan, 2008; Ranjbar et al., 2008). The air-lift-type photobioreactor with the centric tube could provide a regular circulation of the culture in that the air rising from the inner column made the circulating liquid flow out of the inner column whereupon it was gravitationally forced downward (as shown in **Figure 3**) (Chisti, 1989; Chisti, 1998). The regular circulation of the culture resulted in a more effective mixing for growth (Oncel and Sukan, 2008). Ranjbar et al. (2008) also reported that the light regime inside a photobioreactor could be improved and a high-density growth was attainable by using an air-lift-type photobioreactor. In our designed photobioreactor, there are perforations of 5 mm diameter regularly distributed along the porous centric tube. A similar concept has also been reported in which the liquid flowing through the rising zone could horizontally flow through the perforations (Xu et al., 2008). The result indicated that the growth of *Chlorella* sp. NCTU-2 cultured in a photobioreactor with a porous centric tube was even 37% higher than when cultured in a photobioreactor with a centric tube. The perforations along the centric tube in the porous centric-tube photobioreactor could provide a shorter mixing time; therefore, this photobioreactor possesses a better mixing efficiency (Fu et al., 2003). In a high-density culture, the main limitation is the light penetration, which will decrease due to the self-shading effect of the microalgal cells. However, a photobioreactor providing a light/dark zone could minimize the self-shading effect (Chae et al., 2006). The perforations along the centric tube could increase the frequency with which the microalgal cells are exposed to light/dark cycles. The more frequent light/dark cycles affect the productivity and the yield of biomass and have been reported to lead to higher growth and photosynthesis rates (Hu and Richmond, 1996; Nedbal et al., 1996).

6.2.2 CO_2 removal of Chlorella sp. NCTU-2 in the photobioreactors

The CO_2 removal efficiency of *Chlorella* sp. NCTU-2 cultivated in the three types of photobioreactor was evaluated and compared. The microalgal cells were cultured in batch cultures. When the biomass concentration reached 3 g L^{-1}, the microalgal cells were centrifuged and resuspended in fresh medium. Then, the microalgal cells were divided into equal parts and cultured in the photobioreactors at a biomass concentration of approximately 2 g L^{-1} and with 5% CO_2 aeration at 0.25 vvm. The CO_2 removal efficiency was determined by measuring the influent load and the effluent load. **Table 9** shows a comparison of the CO_2 removal efficiencies of *Chlorella* sp. NCTU-2 cultivated in photobioreactors without inner column, with centric-tube and with porous centric-tube column. The results show that the CO_2 removal efficiency in the porous centric-tube photobioreactor is 45 and 52% higher than those in the bubble column and centric-tube photobioreactors, respectively. The CO_2 removal efficiency in the porous centric-tube photobioreactor showed the highest efficiency of CO_2 removal, which is probably due to the higher mixing efficiency and the higher photosynthetic rate. A similar result was also reported by Xu et al. (2008) that the quality of mixing is critical for the performance of a bioreactor, and a shorter mixing time was obtained with an air-lift reactor with a net draft tube, which also provided a horizontal flow, in comparison with the mixing time in a bubble column reactor and an air-lift reactor without a net draft tube. The result has also been confirmed by Grobbelaar (1994) who indicated that higher mixing resulting in more frequent light/dark cycles would enhance the photosynthetic efficiency.

6.2.3 Biomass productivity of Chlorella sp. NCTU-2 in semicontinuous cultivation

Before the operation of semicontinuous cultivation, the microalgal cells were cultivated in a porous centric-tube photobioreactor. When the cell density reached approximately 3 g L^{-1} (beginning of the early stationary phase), the mode was changed to fed-batch cultivation in that the feed medium was only supplied with 750 mg $NaNO_3$ and 44.11 mg $NaH_2PO_4 \cdot H_2O$ per liter every 2 days (as shown in **Figure 10**). After 6 days of cultivation, the biomass concentration reached approximately 5 g L^{-1}. This almost corresponds to the maximum biomass concentration of a *Chlorella* sp. NCTU-2 culture grown in the porous centric-tube photobioreactor at a high growth rate. The growth potential would be decreased due to the decreasing light utilization at the higher biomass concentration of the culture. For the

maintenance of the high-density culture and of biomass production, the semicontinuous culture mode was then applied. For the semicontinuous culture mode, the growth profiles of *Chlorella* sp. NCTU-2 cultured in the centric-tube photobioreactor aerated with 5% CO_2 and operated with 1/4 (i.e. one fourth of the volume of the culture broth was replaced by fresh medium at intervals of 2 d), 1/3 (one third of the broth replaced at 3-day intervals) or 1/2 (one half of the broth replaced at 8-day intervals) replacement are shown in **Figure 11**. A steady-state growth profile was seen with each broth replacement during semicontinuous culture. The steady-state growth profile indicated that continuous growth of the microalgae cultivated in the porous centric-tube photobioreactor could be sustained in a high-density culture.

Table 10 shows the performance of the broth replacement strategies in the semicontinuous culture mode. At a high culture density (biomass concentration from 2.47 to 4.94 g L^{-1}), the specific growth rates and biomass productivities in the 1/4, 1/3 and 1/2 replacement were 0.106, 0.118 and 0.132 day^{-1}, and 0.61, 0.53 and 0.51 g L^{-1} d^{-1}, respectively. Compared with our previous study (Chiu et al., 2008), the biomass productivity in the maintained semicontinuous culture mode was 0.458 g L^{-1} d^{-1}. The results indicate that, for the porous centric-tube photobioreactor in semicontinuous culture mode, the maximum biomass productivity was 0.61 g L^{-1} when one fourth of the culture broth was recovered from the culture every 2 d.

6.2.4 CO_2 removal efficiency at a variety of culture densities at different aeration rates

For the study of CO_2 removal, microalgal cells were collected and cultured in a biomass concentration range from 1.03 to 5.15 g L^{-1}. Different culture densities were obtained by condensing the microalgal cells by centrifugation. The microalgal cells were resuspended in fresh medium for further experiments. All the cultures at a variety of microalgal densities were provided with premixed 10% CO_2 gas at different aeration rates. The CO_2 removal efficiency was evaluated by measuring the influent load and effluent load airstreams at different aeration rates and cell densities of the microalgae. **Figure 12** shows the correlation between CO_2 removal efficiency, culture aeration rate and biomass concentration. The regression lines and the equations were as follows: $y_1=0.1204x+0.0295$ for 0.125 vvm, $y_2=0.108x-0.0308$ for 0.25 vvm, and $y_3=0.0483x-0.0223$ for 0.5 vvm. Here, the value of y_1 is the CO_2 removal efficiency (%) and the value x is the biomass concentration (g L^{-1}).

The result indicates that an increasing CO_2 removal efficiency could be achieved by a lower aeration rate. Also, the CO_2 removal efficiency could be increased by cultivation at a high density. Mandeno et al. (2005) have revealed that the CO_2 removal efficiency decreased with increasing gas flow. This may have resulted from bubble coalescence. The amount of coalescence would have increased with the increased gas flow rate. As the bubbles coalesce, the bubble surface area per unit gas volume would decrease and the larger bubbles would rise faster than the smaller ones. Moreover, CO_2 absorption from the bubbling gas would also decrease with decreasing surface area per unit gas volume of the bubbles. In order to obtain a higher CO_2 removal efficiency, high-density cultivation was performed because, under these conditions, more CO_2 was consumed by the microalga *Chlorella* sp. NCTU-2. Furthermore, an increase in CO_2 removal efficiency during high-density cultivation may also result from the high-density culture broth causing a higher viscosity, which would in turn increase the gas retention time for CO_2 absorption. The optimal conditions for CO_2 removal in this study were culturing *Chlorella* sp. NCTU-2 at a high biomass concentration of 5.15 g L^{-1} and aeration of the culture at 0.125 vvm. The maximum efficiency of CO_2 removal was 63% (with 10% CO_2 in the aeration gas). Keffer and Kleinheinz (2002) have demonstrated that the maximum CO_2 reduction was 74% with *Chlorella vulgaris* cultured in a bubble column photobioreactor, but the CO_2 concentration for aeration was 100-fold lower than that in this study. In this study, the CO_2 removal efficiency could still reach 63% when using the porous centric-tube photobioreactor with a high-density culture, although the CO_2 concentration for aeration was 100-fold higher than that reported by Keffer and Kleinheinz (2002). Compared with our previous study (Chiu et al., 2008), due to the achievement of a high-density culture in the porous centric-tube photobioreactor, the CO_2 removal efficiency was increased from 20 to 53% at the same aeration rate and CO_2 concentration. By using the porous centric-tube photobioreactor with semicontinuous cultivation strategy, a high culture density could be maintained, up to approximately 5 g L^{-1}; besides, a high efficiency of CO_2 removal could be achieved.

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

7.1 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). CO_2 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 CO_2 emissions from energy use (Kadam, 2002), and steel plants are the single largest source of energy-related CO_2 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 CO_2 fixation rates than terrestrial plants and no requirement for further disposal of the trapped CO_2. The incorporation of CO_2 into a biomass carbon source, such as carbohydrates and lipids, by microalgal fixation of CO_2 by photosynthesis is the most promising potential method for CO_2 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 CO_2 from 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.

In general, the primary emission in 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 (Negoro et al., 1991; 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℃ due to irradiation by sunlight. Feasibility of sequestering CO_2 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 CO_2-tolerant mutant strain of *Chlorella* sp. was used in an on-site outdoor microalgal cultivation with flue gas aeration. The flue gas was generated 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 CO_2, NO and SO_2 from the flue gas were evaluated.

7.2 Results and discussion

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

7.2.1.1. 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 CO_2 (> 10% CO_2). Given the high concentration of CO_2 in flue gas (about 20-25% CO_2), 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℃ 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.

Figure 13 shows the growth curves of *Chlorella* sp. (wild-type, WT) and *Chlorella* sp. MTF-7 aerated with flue gas or CO_2-enriched gas (2, 10, or 25% CO_2 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 CO_2. The maximum biomass concentrations in *Chlorella* sp. MTF-7 cultures aerated with 2, 10 or 25% CO_2 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% CO_2 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% CO_2, 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% CO_2, 4% O_2, 80 ppm NO and 90 ppm SO_2, was higher than that of the cultures aerated with 2, 10 or 25% CO_2-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 CO_2 provided to the control culture was the same as the average concentration in the flue gas (11%). However, the growth rate of *C. vulgaris* cultures aerated by flue gas from an incinerator was 48% higher than that of the control culture. The high concentration of CO_2 in flue gas was a major factor in microalgal growth (Yoo et al., 2009). In our previous study, a high initial density of *Chlorella* sp. could overcome the environmental stress induced by high CO_2 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 CO_2 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 NO_2^- and then oxidized to NO_3^-, 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 CO_2 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 SO_2. Lee et al. (2000) reported that the growth of *Chlorella* KR-1 aerated with simulated flue gas containing 150 ppm SO_2 was suppressed because of cellular toxicity when a 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 caladrium*, 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; Kauck 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. **Figure 14** shows the growth curves of *Chlorella* sp. WT and *Chlorella* sp. MTF-7 when aerated with flue gas at 25, 30, 35 or 40℃. The average growth rates of the *Chlorella* sp. WT cultures that were aerated with flue gas at 25, 30, 35 or 40℃ were 0.23, 0.21, 0.14 and 0.11 g L^{-1} 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℃ were 0.37, 0.39, 0.32 and 0.24 g L^{-1} d^{-1}, respectively. The optimal growth temperature for *Chlorella* sp. MTF-7 was 30℃, and the maximum biomass productivity of *Chlorella* sp. MTF-7 cultured at 30℃ and aerated with flue gas was 0.70 g L^{-1} d^{-1}. However, the growth rate and biomass productivity of *Chlorella* sp. MTF-7 that was cultured at higher temperatures (35 and 40℃) remained high and were significantly greater than those of *Chlorella* sp. WT, even when the

wild-type microalgal cells cultured at 25 and 30℃.

7.2.1.2. 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 (**Figure 15**). 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 (**Figure 16**). 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.

Figure 17 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 (**Figure 17A**) and intermittent flue gas (**Figure 17B**) 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 g L^{-1} d^{-1} with continuous flue gas aeration, and 0.30, 0.36, 0.29 and 0.28 g L^{-1} 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 g L^{-1} d^{-1}, and the average biomass growth rate was 0.36 g L^{-1} 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.

7.2.2. Flue gas bioremediation by continuous flue gas aeration

7.2.2.1. CO_2 removal

For the on-site bioremediation of CO_2 in flue gas from a coke oven, the time course of the CO_2 removal efficiency of the microalgal cultures that were aerated with flue gas was 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 by CO_2 gas sensors. **Figure 18A** illustrates the inlet load, outlet load and CO_2 removal efficiency of *Chlorella* sp. MTF-7 under flue gas aeration. The CO_2 removal efficiency was 95% at 10 min after flue gas introduction and 50% after 25 min of flue gas aeration. The decrease in the CO_2 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% CO_2 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 CO_2 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 CO_2 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 CO_2 removal efficiency, the CO_2 removal efficiency was approximate 20% (Chiu et al., 2009b). Moreover, this phenomenon is also confirmed in previous study that the CO_2 removal efficiency was remarkably consistent and showed a stable pattern from outlet CO_2 measurements (Chiu et al., 2008).

7.2.2.2. NO and SO_2 removal

The time courses of the NO and SO_2 removal efficiencies of the microalgal cultures that were aerated with flue gas were also determined. **Figure 18B** 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 SO_2 concentrations in the inlet and outlet loads of the flue gas were 87 ± 9 and 37 ± 7 ppm, respectively, and the SO_2 removal efficiency of the *Chlorella* sp. MTF-7 cultures under flue gas aeration was approximately 55% (**Figure 18C**). Nagase et al. (2001) have reported that, in addition to reducing CO_2 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 CO_2, NO and SO_2 capture from the flue gases. To make the biological CO_2, NO and SO_2 capture process to be economically feasible, the utilization of the produced microalgal biomass is a critically important issue.

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

7.2.3.1. CO_2 removal

To improve the efficiency of CO_2 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 CO_2 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 CO_2 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 (**Figure 19**). The average CO_2 removal efficiencies at the 20, 30 or 40 min time intervals were 70, 63 and 45%, respectively.

To evaluate the system stability for CO_2 removal during intermittent flue gas aeration, the intermittent flue gas aeration was operated with a 30-min time interval. CO_2 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 (**Figure 20A**). The pattern of the changes in the pH of the *Chlorella* sp. MTF-7 culture was stable during intermittent flue gas aeration (**Figure 20B**). 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 CO_2 in the microalgal culture broth also followed a repetitive pattern during the gas-switching aeration cycles (**Figure 20C**). During flue gas aeration, the pH decreased, and the dissolved inorganic carbon (DIC) concentration increased because of CO_2 absorption. During air aeration, the pH increased, and the DIC concentration decreased, implying that the increase in the outlet load of CO_2 contributed to

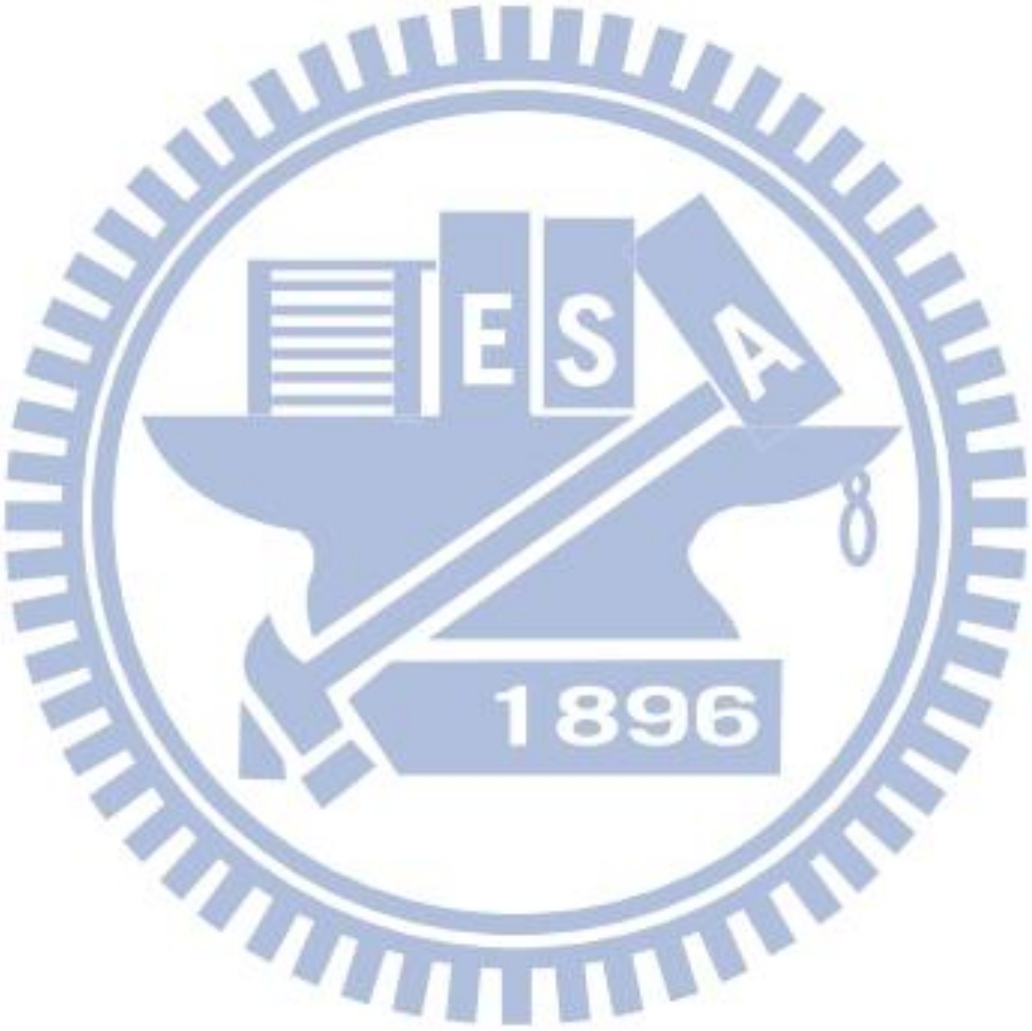
the pH decrease. Our system was operated for 9 cycles during the day, and the patterns of the fluctuations in the values of pH, DIC and CO_2 removal efficiency were stable throughout (data not shown).

7.2.3.2. NO and SO_2 removal

The NO and SO_2 removal efficiencies were also evaluated during the intermittent flue gas application. **Figure 21A** 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. **Figure 21B** illustrates the patterns of the SO_2 concentrations of the inlet and outlet loads when *Chlorella* sp. MTF-7 was aerated with intermittent flue gas. The SO_2 removal efficiency was also stable, and the average SO_2 removal efficiency reached approximately 50%.

To determine whether CO_2, NO and SO_2 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 (**Figure 16**). 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 CO_2, NO and SO_2 concentrations of the inlet and outlet loads, the average CO_2, NO and SO_2 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, CO_2, NO and SO_2 could be constantly removed from the flue gas. The CO_2, NO and SO_2 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.



8. Conclusions

In this study, we establish an outdoor photobioreactor system for microalgal biomass production. The study is divided into four parts: 1. The strategy of increasing CO_2 tolerance (> 15% CO_2) and cell density in the microalgal cultures was performed in this study. 2. The semicontinuous operation strategy enhanced the microalgal biomass production. 3. We also demonstrated the correlation between CO_2 removal efficiency, culture aeration rate and culture density by using porous centric tube photobioreactor. 4. We demonstrated an outdoor photobioreactor aerated with flue gas for on-site bioremediation. The conclusions are showed in below, respectively.

8.1 Part I. Reduction of CO_2 by a high-density culture of *Chlorella* sp. in a semicontinuous photobioreactor

1. In previous studies, researchers focused on the effects on high concentration of CO_2 aeration for microalgae growth but low concentration of CO_2 aeration (< 2% CO_2) for CO_2 removal efficiency. In our study, CO_2 removal efficiency in the microalgal cultures aerated with higher CO_2 concentration was evaluated. Our data show that the CO_2 removal efficiency in the developing microalgal photobioreactor at 2%, 5%, 10% and 15% CO_2 aeration could reach to 58%, 27%, 20% and 16%, respectively.
2. The strategy of increasing CO_2 tolerance and cell density in the microalgal cultures was performed in this study. At the initiating stage of culture, the microalgal cells were grown and adapted to an enriched- CO_2 (2%) environment. When a high-density microalgal culture reached, the culture could be executed under 10% or 15% CO_2 aeration without decreasing performance of cell maintenance and growth. It is valuable information on the use of CO_2 reduction system of microalgal photobioreactor.
3. It is also confirmed that CO_2 reduction system could be extended to multiple units in parallel photobioreactor for discharging waste gas in a large scale. The amount of lipid and biomass production and CO_2 removal efficiency in the individual parallel photobioreactor and in the single photobioreactor also were similar.

8.2 Part II. Lipid accumulation and CO_2 utilization of *Nannochloropsis oculata* in response to CO_2 aeration

1. The lipid accumulation from microalgae with different concentration of CO_2 aeration

would be evaluated for the efficiency of biodiesel production. In our study, CO_2 utilization for microalgal lipid accumulation in the cultures aerated with different concentration of CO_2 was evaluated. Our data show that the CO_2 utilization for microalgal lipid accumulation at low concentration of CO_2 aeration would be more potential and more efficient.

2. The CO_2-tolerance for microalgae is an important issue in the use of waste flue gas as a carbon source. *N. oculata* grew well at 2% CO_2 aeration, but completely inhibited at 5, 10, and 15% CO_2 aeration. After a pre-adapting culture and applying to semicontinuous system, *N. oculata* could grow well at high CO_2 aeration. The growth profile is similar and could be applied to CO_2 removal system.

3. We demonstrate the strategy of increasing total lipid production for biodiesel production. The comparison of productive efficiencies in semicontinuous system was performed with half (for one-day) or three fifth (for three-day) replacement culture approaches. Our data show the culture broth being daily replacement could be more efficient not only for the biomass production but also for lipid production. It is valuable information that the microalga cultured in the semicontinuous operation system could efficiently introduced 2% CO_2 aeration for biomass and lipid production in the long-term operation.

8.3 Part III. The air-lift photobioreactors with flow patterning for a high-density culture of microalgae and carbon dioxide removal

1. In our study, a porous centric tube photobioreactor was designed for microalgal culture. The comparison of the growth of *Chlorella* sp. NCTU-2 cultivated in the photobioreactors without inner column, with centric tube and with porous centric tube column was evaluated. Our data show that the maximum optical density and specific growth rate of the cultures in photobioreactors without inner column, with centric tube and with porous centric tube column in a batch culture were 11.5, 12.3 and 16.8 (at A_{682}), and 0.18, 0.226 and 0.252 d^{-1}, respectively. Furthermore, the efficiency of CO_2 removal in the porous centric tube photobioreactor is 45% and 52% higher than those in the bubble column and centric tube photobioreactors, respectively. It is valuable information on the design of microalgal photobioreactor.

2. We demonstrate a strategy of a long-term maintained high-density culture in our designed porous centric tube photobioreactor. *Chlorella* sp. NCTU-2 cultured in the centric tube

photobioreactor and operated by 1/4 (i.e., one-fourth volume of cultured broth was replaced by fresh medium at an interval of 2 days) and 1/3 (one-third broth replaced at 3 days interval) and 1/2 (one-second broth replaced at 8 days interval) replacement. A steady state of growth profile was seen in each broth replacement of semicontinuous culture. The maximum biomass productivity could achieve to 0.61 g L^{-1} in 1/4 of the culture broth recovered from the culture every 2 days.

3. We also demonstrated the correlation between CO_2 removal efficiency, culture aeration rate and culture density by using porous centric tube photobioreactor. The result indicates that increasing of CO_2 removal efficiency could be accomplished by lower aeration rate. The optimal condition for CO_2 removal could achieved in this study was that *Chlorella* sp. NCTU-2 was cultured at high optical density of 25 (A_{682}) and the culture was aerated at 0.125 vvm. The maximum efficiency of CO_2 removal was 63% (the aerated gas with 10% CO_2). According to our best knowledge, this is the first report addressed the efficacy on CO_2 removal with different culture density and aeration rate in a closed microalgal photobioreactor aerated with high (10%) CO_2 concentration.

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

1. In this study, a microalga, *Chlorella* sp. MTF-7, was isolated by chemical mutagenesis. The microalgal strain *Chlorella* sp. MTF-7 grew in the presence of flue gas, and the flue gas temperature was 40-45^{o}C when it was introduced into the microalgal cultures. It is a potential microalgal strain for bioremediation of flue gas.

2. We demonstrated an outdoor photobioreactor aerated with intermittent flue gas for on-site bioremediation. On-site bioremediation of CO_2, NO and SO_2 in flue gas by the *Chlorella* sp. MTF-7 cultures could reach approximately 60%, 70% and 50%, respectively. According to our best knowledge, this is the first report addressed and evaluated on-site bioremediation of CO_2, NO and SO_2 in flue gas.

3. We also demonstrated a double-set of photobioreactor systems alternately aerated with flue gas for continuous flue gas bioremediation. An on-site outdoor photobioreactor system using a gas-switching cycle operation could remove a high percentage of the CO_2, NO and SO_2 from flue gas generated from a coke oven. We believe that it is valuable information for the use of flue gas bioremediation system of microalgal cultures.

9. Future works

In this study, we screened and isolated a thermal- and CO_2-tolerant microalgal strain and establish an outdoor photobioreactor system for microalgal biomass production. We also established the strategies of increasing CO_2 tolerance (> 15% CO_2) and high density cultivation. In addition, the semicontinuous operation strategy enhanced the microalgal biomass production. We demonstrated an outdoor photobioreactor aerated with flue gas for on-site bioremediation. We concluded that the isolated thermal- and CO_2-tolerant microalgal strain, *Chlorella* sp. MTF-7 is a potential strain for on-site bioremediation of CO_2, NO and SO_2 from flue gas. However, there are still many technical bottlenecks for improving the on-site bioremediation from flue gas by microalgae.

The technical bottlenecks and comments are as followings: 1) the operation of subculture should be simplified for reducing the cost of electricity: combination of serial and parallel connection of photobioreactors into a module system and using the gravity force for broth subculture are potential strategies; 2) the cost of photobioreactor is still a limiting factor for up-scaling: the configuration of photobioreactor could replace by plastic bag with holders or cheap and solid plastics; 3) the manual control of gas aeration in the photobioreactor system could be applied a sensor system for automatic control: the flue gas aeration drop down the pH value, thus the pH could be an indicator for intermittent gas aeration.

Actually, the major limitation of reducing the cost of the microalgal technology is the downstream process. Due to the size of microalgae, the microalgae harvesting and oil extraction for biodiesel production are the major bottlenecks for sustainable energy development from microalgal technology. The integrated process of biorefinery is an important route. The microalgal biomass utilization should be extremely perfect. In addition, for bioenergy consideration, microalgal biomass for biofuel production should be a side product for maximized the microalgal biomass utilization. The microalgal biomass should be mainly used for high value product production and the residues could be utilized for bioenergy production, such as cellulose for bioethanol production and the oil for biodiesel production. It is the potential route for bioenergy production from microalgal technology.

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Table 1. Oil productivity of different energy plants.

Oil-Producing Crops	Oil production (L/ha/years)	Relative oil productivity	Land area needed (M ha) [a]	Percent of existing US cropping area [a]
Oil Palm	5950	5	45	24
Coconut	2689	2.26	99	54
Jatropha	1892	1.59	140	77
Rapeseed/Canola	1190	1	223	122
Peanut	1057	0.89	259	144
Sunflower	954	0.8	287	159
Safflower	776	0.65	353	196
Mustard	571	0.48	480	267
Soybean	446	0.38	594	326
Corn(Maize)	172	0.14	1540	846
Microalgae[b]	136,900	115.04	2	1.1
Microalgae[c]	58,700	49.33	4.5	2.5

[a] For meeting 50% of all transport fuel needs of the United States.
[b] 70% oil (by wt) in biomass.
[c] 30% oil (by wt) in biomass.

Oil crops can not significantly contribute to replacing petroleum derived liquid fuels in the foreseeable future. If microalgae are used to produce biodiesel, only 1% and 2.5% of the total U.S. cropping area can be sufficient for producing algal biomass that satisfies 50% of the transport fuel needs of U.S. (Pahl, 2005; Chisti, 2007).

Table 2. Advantages and disadvantages of open and closed culture systems

	Closed system	Open system
Basic type	Tubular Coil Column Flat plate	Raceway-shape Open pond Open pond with rotating arm
Operation regime	Batch Fed batch Continuous Semi-continuous	Batch Semi-continuous
Contamination risk	Extremely high	Low
Sterility	Achievable	None
Space required	Low	High
Mixing	Uniform	Very poor
Water-losses	Almost none	Extremely high
CO_2-losses	Almost none	High
Biomass quality	Susceptible	Not susceptible
Area/volume ratio	High (20 – 200 m^{-1})	Low (5 – 10 m^{-1})
Population density	High	Low
Process control	Easy	Difficult
Investment	High	Low
Operation costs	High	Low
Light utilization efficiency	Excellent	Poor
Temperature control	More uniform temperature	Difficult
Reproducibility of production parameters	Possible within certain tolerances	Dependent on exterior conditions
Standardization	Possible	Not possible
Weather dependence	Insignificant, because closed configurations	Absolute, production impossible during rain

	allow production also during bad weather	
Productivity	3 – 5 times more productive	Low
Gas transfer control	High	Low

Summarized from Ugwu et al., 2008 and Mata et al., 2010.

Table 3. Productivity of biomass grown outdoors in the various photobioreactors

Basic type	Orientation	Inside diameter (cm)	Location	Strain	Highest productivity		Reference
					($g/m^2/d$)	(g/L/d)	
Open pond	---	13-15	Israel	*Spirulina platensis*	27	0.18	Richmond et al., 1990
Open pond	---	1	Czech	*Chlorella* sp.	25	2.50	Doucha and Livansky, 1995
Raceway pond	---	20	France	*Chlorella* sp.	13.2		Hase et al., 2000
Raceway pond	---	20	France	*Chlorophyta* sp.	8.2		Hase et al., 2000
Tubular	Vertical	5	Germany	*Chlorella* sp.	35.7		Pulz, 2001
Tubular	Horizontal	12.3	Italy	*Spirulina maxima*	25.0	0.25	Torzillo et al., 1986
Tubular	Horizontal	2.6	Italy	*Spirulina* sp.	27.8	---	Torzillo et al., 1986
Tubular	Horizontal	2.5	Israel	*Spirulina platensis*	27.0	1.60	Richmond et al., 1993
Tubular	Horizontal	2.6	Spain	*Isochrysis galbana*	---	0.32	Grima et al., 1994
Tubular	Horizontal	6.0	Spain	*Phaeodactylum*	---	2.02	Fernandez et al., 1998
Tubular	Horizontal	3.0	Spain	*Phaeodactylum*	---	2.76	Grima et al., 1996
Tubular	Horizontal	6.0	France	*Porphyridium cruentum*	25.0	0.36	Chaumont et al., 1988
Tubular	Horizontal	41		*Haematococcus pluvialis*	13		Olaizola, 2003

Continued

Basic type	Orientation	Inside diameter (cm)	Location	Strain	Highest productivity		Reference
					($g/m^2/d$)	(g/L/d)	
Tubular	Inclined	2.5	Singapore	*Chlorella pyrenoidosa*	72.5	2.90	Lee et al., 1995
Tubular	Inclined	1.2	Singapore	*Chlorella pyrenoidosa*	130.0	3.64	Lee and Low, 1991
Tubular	Helical	2.5		*Chlorella* sp.	28.1		Morita et al., 1998
Tubular	Horizontal		Japan	*Chlorococum littorale*	20.5	0.146	Sato et al., 2006
Tubular	Horizontal		Japan	*Chaetoceros calcitrans*	37.3	0.266	Sato et al., 2006
Coil	Vertical	2.4	Australia	*Tetraselmis chuii*	---	1.20	Borowitzka, 1997
Column	Vertical	20.0	Spain	*Phaeodactylum*	---	0.69	Miron et al., 1999
Column	Vertical	2.6	Israel	*Isochrysis galbana*	---	1.60	Hu and Richmond, 1994
Column	Vertical	40	Italy	*Tetraselmis suecica*	38.2	0.42	Zittelli et al., 2006
Column	Vertical	16	Taiwan	*Chlorella* sp.		0.52	Chiu et al., 2011
Column	Inclined	3.5	Italy	*Nannochloropsis* sp.	52.5		Zittelli et al., 2003
Flat plate	Vertical	1.5	Japan	*Synechocytis aquatilis*	31.0		Zhang et al., 2001

Flat plate	Vertical	10		*Nannochloropsis* sp.	12.0		Zhang et al., 2001
Flat plate	Vertical		Italy	*Nannochloropsis* sp.		0.36	Rodolfi et al., 2009
Flat plate	Inclined	0.6		*Chlorella* sp., strain P12	22.8		Doucha et al., 2005
Flat plate	Inclined	10.4	Israel	*Spirulina platensis*	33.0	0.30	Hu et al., 1996
Flat plate	Inclined	1.3	Israel	*Spirulina platensis*	51.0	4.30	Hu et al., 1996
Flat plate	Inclined	3.2	Italy	*Spirulina platensis*	24.0	0.80	Tredici et al., 1991

Table 4. The main international companies that using microalgal cultivation for CO_2 reduction and bioerergy development.

Companies	Country	Industrial brief introduction	Additional remark
Cyanotech	USA	Microalgal biomass supplied for health food and food supplement	In recent years, the company will also conclude the algae technology as a biomass energy research and development priorities.
HR BioPetroleum (HRBP)	USA	Using marine microalgae for bioenergy production and developing the technique for CO_2 reduction from industries	Netherlands Shell Oil company and Cellena are the joint venture and invest $ 7 billion to carry out the study of microalgae bio-diesel technology.
PetroSun	USA	Using algal farming for microalgal oil production	In about 1,100 acres of algae farming, PetroSun expects to produce 4.4 million gallons of algal oil, and declared the establishment of 5 million tons/year production capacity.
Solazyme	USA	Biodiesel production by bio-enzymatic strategy	In recent years, with the second-largest U.S. oil company Chevron co-financing of approximately $ 45 million project to produce oil from the algae million gallons of algae biodiesel.
Sapphire Energy	USA	Transesterification technique for microalgal oil	Successfully produced the 91 # gasoline from algae fit in with American society of testing and material certification standard. The microalgal bio-fuels involved in its aircraft, Boeing 737-800 flight trial.
OriginOil	USA	One step microalgal oil extraction	Cooperated with the Australian energy company, MBD, in 2010, and providing a single step method for algae oil extraction (Single-Step Extraction System) This technology is expected to reduce the cost of microalgae harvesting process and the cost of energy.
Valcent Products Inc,.	USA、Canada	Famous by development of vertically vegetables cultivation	Using the material of plastic bag for microalgae indoor culture system, the production cost of algae oil can be reduced to $ 0.8 per gallon, while the area per acre per year to produce 33,000 gallons of algae oil.
Aquaflow	New	Extraction and	Cooperated with Boeing (Boeing) to

Bionomic Corporation	Zealand	purification of biodiesel from the sewage treatment of microalgal biomass	produce jet fuel (Jet-fuel)
AlgaeLink	Netherlands	The photobioreactors equipment plant	Production of diameter 25 cm, total length of 480 meters and 2,000 meters, two kinds of horizontal cylindrical microalgal photobioreactor.
BioKing	Netherlands	The biodiesel equipment plant	The AlgaeLink sister plant for biodiesel production
Seambiotic	Israel	Using algal farming for microalgal oil production	Plans to operate a microalgal farm next to a power plant in Israel.
LiveFuels	USA	The microalgal integrated system development and research	The use of low-cost natural systems and a rich with eutrophic water for microalgae open pond cultivation. Algal biomass is used for fish food as a natural recovery to achieve low-cost recovery purposes.
General Atomics	USA	Development and Research on microalgal biofuels	Currently, the company signed a $ 43 million contract to develop efficient, low-cost algae aviation fuel production process with the U.S. Defense Advanced Research Projects Agency (DARPA)

Table 5. The biomass production and the specific growth rate of the low- and high-density inoculums of *Chlorella* sp. growth depending on different concentrations of CO_2 aeration.

CO_2 aeration	Biomass (cell dry weight, g L^{-1}) [b, c]	Specific growth rate (μ, d^{-1}) [d]
Low-density inoculum (8×10^5 cells mL^{-1})		
Air [a]	0.537 ± 0.016	0.230
2%	1.211 ± 0.031	0.492
5%	0.062 ± 0.027	0.127
10%	0.010 ± 0.003	-
15%	0.009 ± 0.001	-
High-density inoculum (8×10^6 cells mL^{-1})		
Air	0.682 ± 0.007	0.248
2%	1.445 ± 0.015	0.605
5%	0.899 ± 0.003	0.343
10%	0.106 ± 0.001	-
15%	0.099 ± 0.001	-

[a] CO_2 concentration in the air is around 0.03%.

[b] Biomass was measured when the cells grew up to the plateau stage in the culture.

[c] Each data indicates the mean ± SD, which were measured from three independent cultures.

[d] Specific growth rate was obtained on the exponential logarithmic growth phase by day during the cultivation. The exponential logarithmic growth phase was from 1 to 2 days batch culture with different concentrations of CO_2 aeration.

Table 6. Recovery of lipid and biomass production of the *Chlorella* sp. as waste broth in the semicontinuous photobioreactor under different concentrations of CO_2 aeration compared with single and six-parallel photobioreactor.

CO_2 aeration	Total lipid production (g d^{-1})		Total biomass production (cell dry weight, g d^{-1})	
	In single photobioreactor [a]	In six-parallel photobioreactors [b]	In single photobioreactor	In six-parallel photobioreactors
2%	0.143 ± 0.020	0.852 ± 0.133	0.422 ± 0.061	2.560 ± 0.312
5%	0.130 ± 0.011	0.773 ± 0.060	0.393 ± 0.040	2.343 ± 0.232
10%	0.124 ± 0.029	0.770 ± 0.131	0.366 ± 0.089	2.154 ± 0.511
15%	0.097 ± 0.007	0.601 ± 0.055	0.295 ± 0.031	1.743 ± 0.144

[a] The culture volume in a photobioreactor is 800 mL. Daily waste broth was 400 mL.

[b] Sum of the production from six units of photobioreactor. The medium volume in each photobioreactor is 800 mL. Sum of daily waste broth was 2,400 mL.

[c] Each data indicates the mean ± SD, which were measured daily from Day 1 to Day 8.

Table 7. Daily recovery of biomass and lipid of *N. oculata* NCTU-3 cultured in the semicontinuous system aerated with different CO_2 concentrations

CO_2 aeration	Total biomass productivity (cell dry weight, g L^{-1} d^{-1})	Total lipid productivity (g L^{-1} d^{-1})	Percentage of lipid content (%)
2%	0.480 ± 0.029	0.142 ± 0.049	29.7 ± 2.0
5%	0.441 ± 0.044	0.113 ± 0.035	26.2 ± 1.9
10%	0.398 ± 0.069	0.097 ± 0.026	24.6 ± 1.7
15%	0.372 ± 0.022	0.084 ± 0.021	22.7 ± 1.9

The semicontinuous cultures were performed for 8 d and a half of broth was replaced each day. The culture volume in photobioreactor is 800 mL. Daily waste broth was 400 mL. Each data indicates the mean ± SD, which were measured daily from Day 1 to Day 8.

Table 8. Biomass and lipid productivity of *N. oculata* NCTU-3 cultured in the semicontinuous system aerated with 2% CO_2 under the treatments of one-day and three-day replacement

Culture	Total biomass productivity (cell dry weight, g L^{-1} d^{-1})	Total lipid productivity (g L^{-1} d^{-1})	Percentage of lipid content (%)
One-day replacement	0.497 ± 0.032	0.151 ± 0.021	30.7 ± 2.4
Three-day replacement	0.296 ± 0.009	0.121 ± 0.035	41.2 ± 1.9

The semicontinuous cultures were performed for 12 d. The cultural broth was replaced by half (for one-day replacement) or three fifth (for three-day replacement) with fresh medium at interval of 24 and 72 h, respectively. The total biomass and lipid productivity were measured from the total replaced broth divided by day. The total replaced broth volume was 4,800 mL in one-day replacement and only 1,920 mL in three-day replacement over 12 d. Each data indicates the mean ± SD.

Table 9. Comparisons of growth potential and CO_2 removal efficiency in the three types of photobioreactors.

	Bubble column	Centric tube	Porous centric tube
Max. biomass concentration (g L^{-1}) [a]	2.369	2.534	3.461
Max. specific growth rate (μ, d^{-1})	0.180	0.226	0.252
CO_2 removal efficiency [b]	24%	23%	35%

[a] The cultures were performed in batch culture with the initial biomass concentration of 1 g L^{-1}.

[b] The comparison of CO_2 removal efficiency between these three types of photobioreactor was operated when the cultured reached to a biomass concentration of approximately 2 g L^{-1} and the cultures were aerated with 5% CO_2 at an aeration rate of 1.0 L min^{-1} (i.e., 0.25 vvm).

Table 10. Performance of the different broth replacement strategies in a semicontinuous cultivation. [a]

Replacement ratio each time [b]	Time interval for each replacement (day)	Recovered volume for each replacement (L)	Specific growth rate (μ, d^{-1})	Biomass productivity ($g\ L^{-1}\ d^{-1}$) [c]
1/4	2	1	0.106	0.61
1/3	3	4/3	0.118	0.53
1/2	8	2	0.132	0.51

[a] All of the cultures were performed in the porous centric tube photobioreactor.

[b] In this semicontinuous cultivation strategy, the initial cultures were cultivated with fed-batch cultures till the cell density was reached to 5 $g\ L^{-1}$. After that, the cultured broth was replaced one-fourth, one-third and one-second by fresh medium, and cultivated till the cell density reached back to 5 $g\ L^{-1}$. The semicontinuous cultures were performed at least for 2 cycles of replacements (at least 15 days) (as shown in **Figure 12**).

[c] Biomass productivity was defined as total recovered biomass divided by reactor working volume and by day.

Figure 1. The typical types of photobioreactors. (A) tubular photobioreactor, (B) flat plate photobioreactor and (C) column photobioreactor。

Figure resources:
(A) Chisti, Y. 2007. Biodiesel from microalgae. Biotechnology Advances 25:294−306.
(B) http://biofuels.asu.edu/
(C) Department of Biological Science and Technology, Po-Ai Campus, National Chiao-Tung University

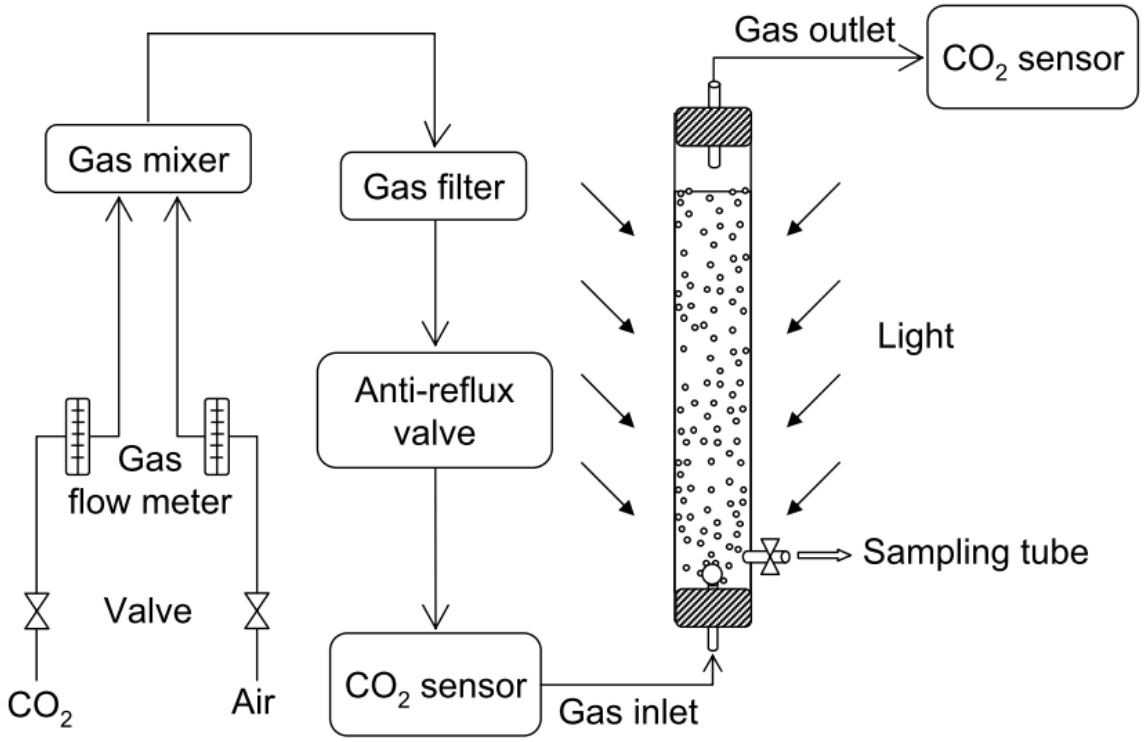

Figure 2. Schematic diagram of the photobioreactor for the experiments on CO_2 reduction for batch and semicontinuous microalgal cultures. The airstreams with different CO_2 concentrations was adjusted by individual gas flow meter and determined via CO_2 sensor. External illumination of light intensity was provided with 300 μmol m^{-2} s^{-1} by a continuous, cool white, and fluorescent light.

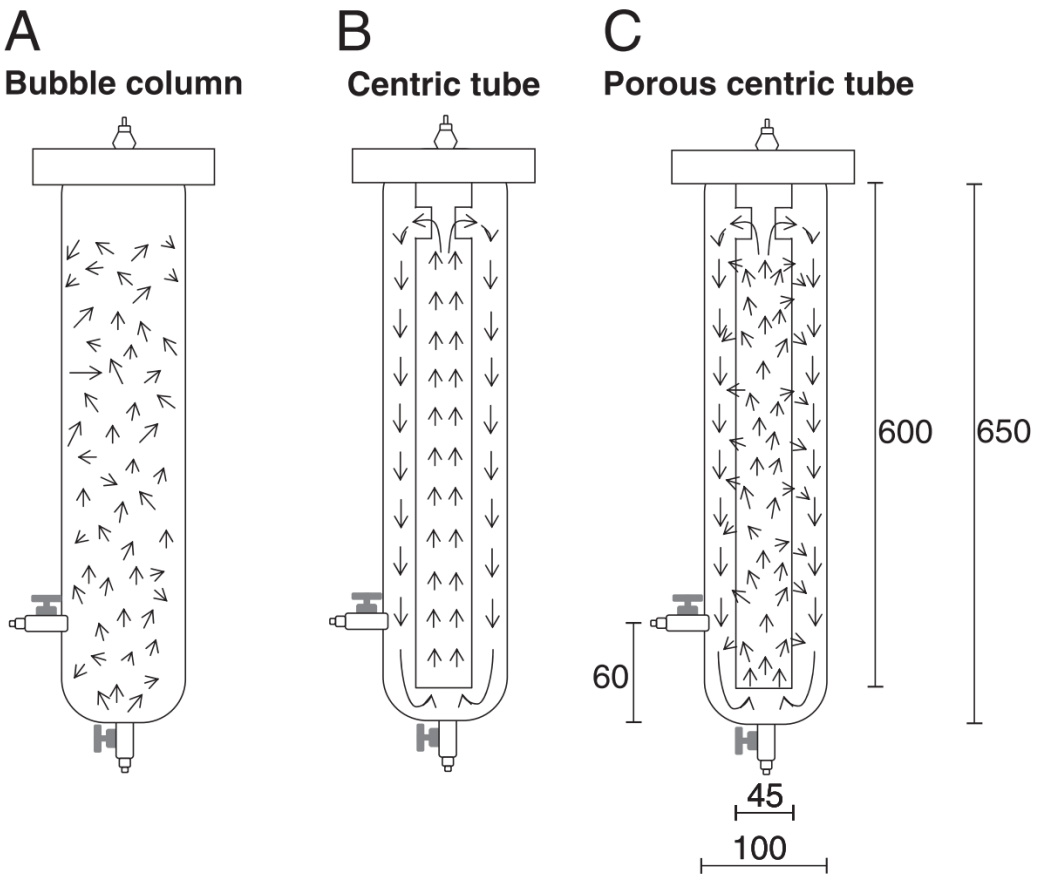

Figure 3. Schematic diagram of the different types of photobioreactor and visualization of the liquid flow patterns. (A) Bubble column-type photobioreactor, (B) centric-tube photobioreactor, (C) porous centric-tube photobioreactor. The flow patterns were visualized by injecting a dye at the liquid surface. The working volume of the photobioreactors was 4 L. Gas was provided and pumped into the photobioreactors *via* a sparger at the bottom of the photobioreactor. External illumination with a light intensity of 300 μmol m^{-2} s^{-1} was provided by a continuous, cool white and fluorescent light source. The arrows indicate the visualized liquid flow patterns.

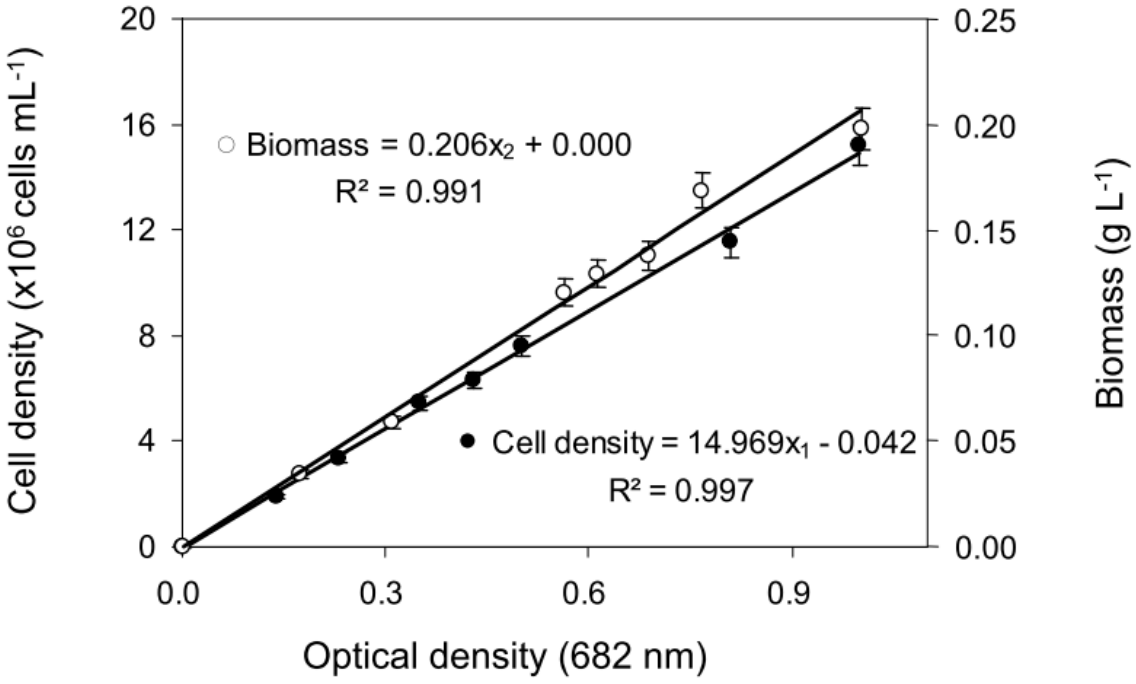

Figure 4. Calibration curves and equations of optical density at A_{682} to the cell density (●) and the biomass (○). Left axis shows optical density *vs.* cell density (cells directed counted by microscope) in the *Chlorella* sp. culture. Right axis shows the optical density *vs.* biomass (g L^{-1}). The calibration curve, correlative equation, and R^2 value of each correlation were indicated. Each point was averaged from three independent measures.

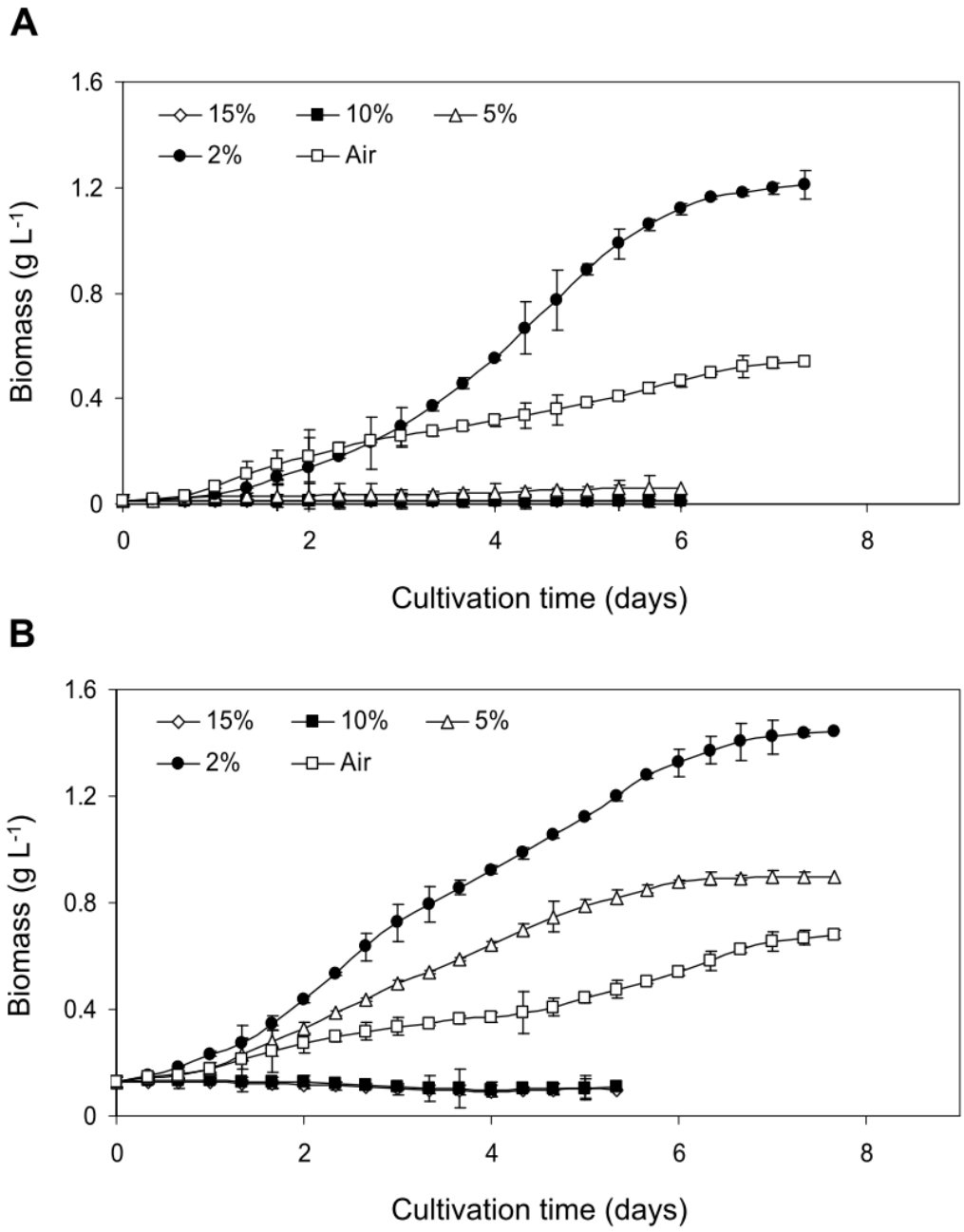

Figure 5. Effects of different concentrations of CO_2 aeration on the growth of *Chlorella* sp.. **A**, shows the growth curve of *Chlorella* sp. inoculated at low-density cells (8×10^5 cells mL^{-1} in an 800-mL cultivation). **B**, shows the growth curve of *Chlorella* sp. inoculated at high-density cells (8×10^6 cells mL^{-1} in an 800-mL cultivation). All experiments were carried out in triplicate. The cultures were illuminated at 300 μmol m^{-2} s^{-1} and bubbled with a flow rate of 0.25 vvm airstreams at 26 ± 1℃.

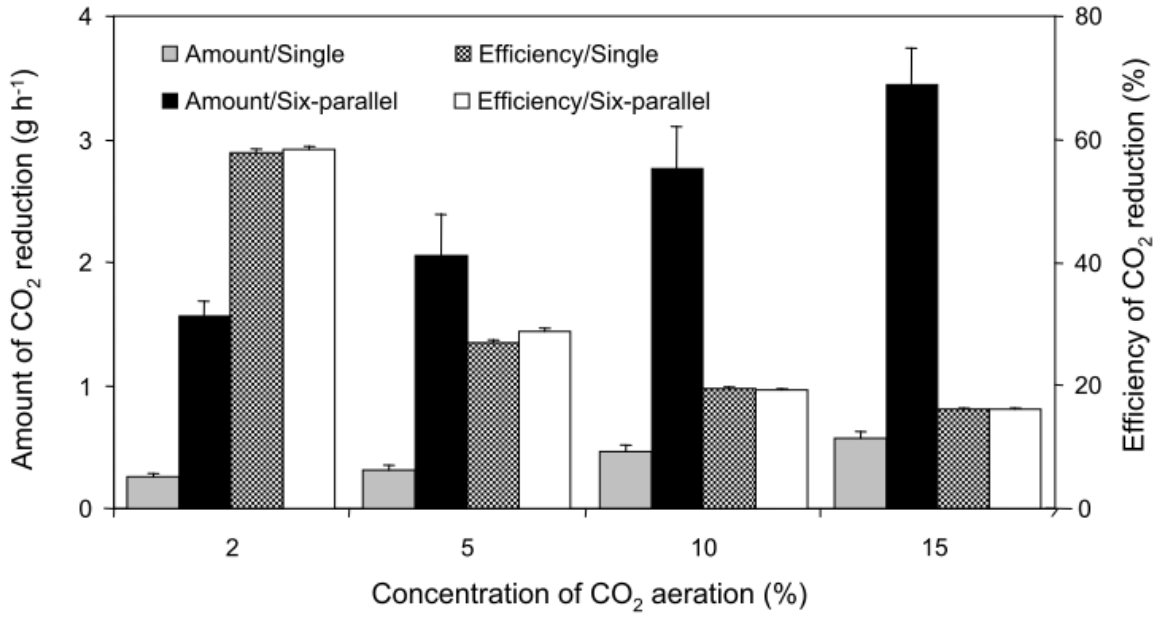

Figure 6. Comparisons of the total amount and efficiency of CO_2 reduction in the single and the six-parallel photobioreactor of semicontinuous *Chlorella* sp. cultures under 2%, 5%, 10%, and 15% CO_2 aeration. Bars show the total amount of CO_2 reduction by single (□) and six-parallel photobioreactor (■) and; bars show the efficiency of CO_2 reduction by single (▩) and six-parallel photobioreactor (□). The total amount and efficiency of CO_2 reduction were determined by the difference of influent and effluent CO_2 loading in airstreams. Each data indicates the mean ± SD and were measured from three independent cultures.

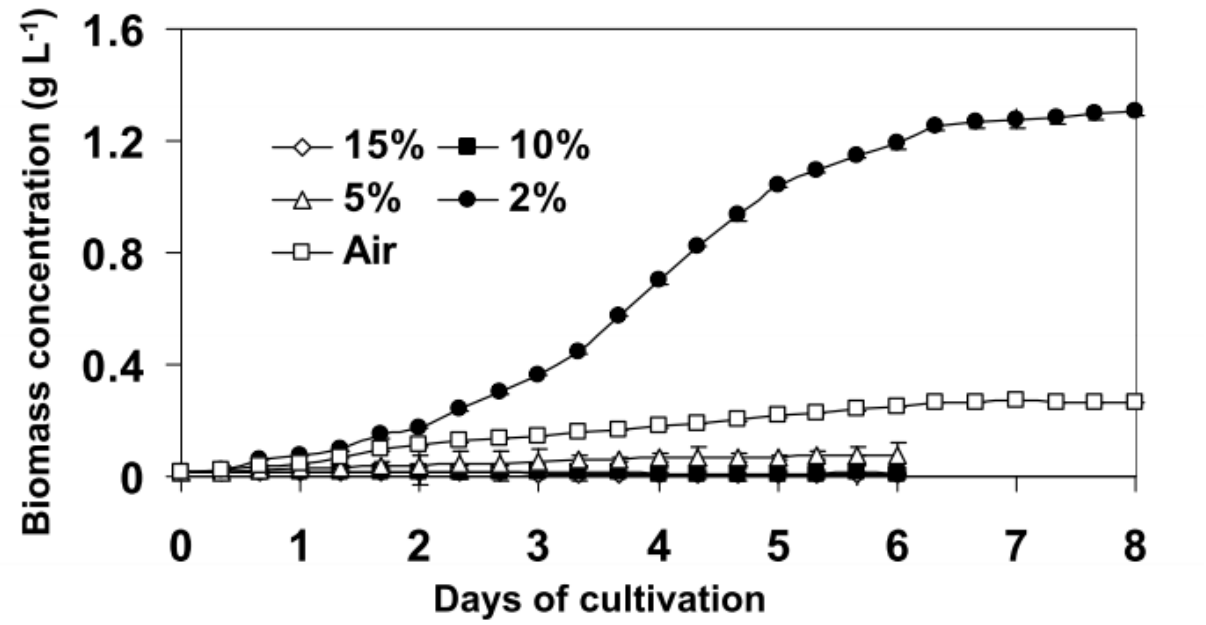

Figure 7. Effect of the concentrations of CO_2 aeration on the growth of *N. oculata* NCTU-3. In the cultures, approximate 0.01 g L^{-1} of microalgal cells was inoculated and cultivated under air, 2, 5, 10, and 15% CO_2 aeration. All experiments were carried out in triplicate. The cultures were illuminated at 300 μmol m^{-2} s^{-1} and bubbled with a flow rate of 0.25 vvm airstream at 26 ± 1℃.

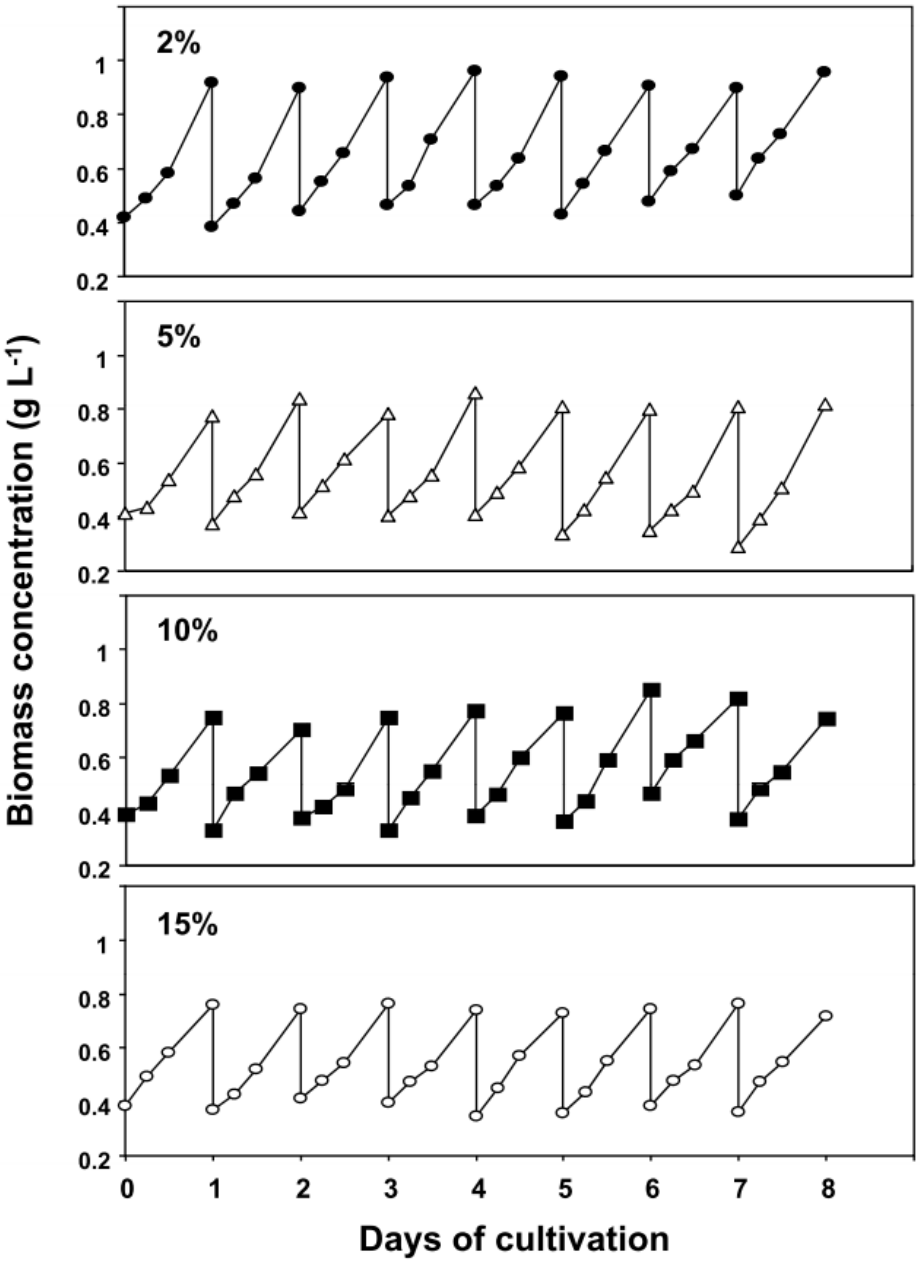

Figure 8. Growth profiles of *N. oculata* NCTU-3 cultured in the semicontinuous system aerated with 2, 5, 10, and 15% CO_2. In the cultures, approximate 0.4 g L^{-1} of microalgal cells was inoculated and cultivated under an illumination at 300 μmol m^{-2} s^{-1} and bubbled with a flow rate of 0.25 vvm airstreams at 26 ± 1℃ for 8 d. Amount of 50% of cultured broth was replaced with the fresh modified f/2 medium at interval of 24 h.

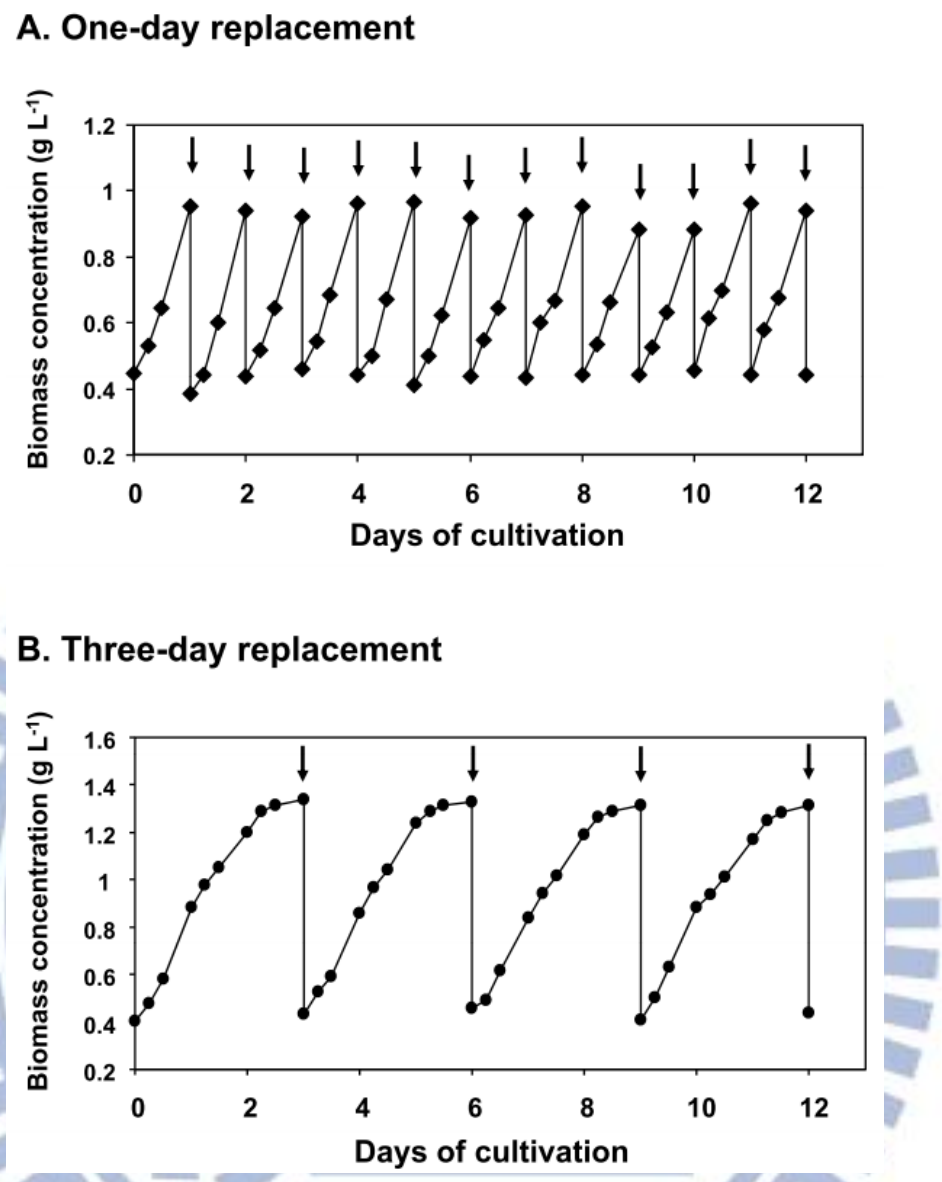

Figure 9. Growth profiles of *N. oculata* NCTU-3 cultured in the semicontinuous system with 2% CO_2 aeration and operated by one-day and three-day replacements. In the cultures, approximate 0.4 g L^{-1} of microalgal cells was inoculated and cultivated under an illumination at 300 μmol m^{-2} s^{-1} and bubbled with a flow rate of 0.25 vvm airstreams at 26 ± 1℃. The cultivations were continuously operated for 12 d. Amount of half and three fifth of cultured broth was replaced with the fresh modified f/2 medium at interval of 24 h (one-day replacement; half broth was replaced each day) and 72 h (three-day replacement; three fifth broth was replaced every 3 d), respectively. The arrows indicate the time when the cultured broth was removed and fresh medium was added.

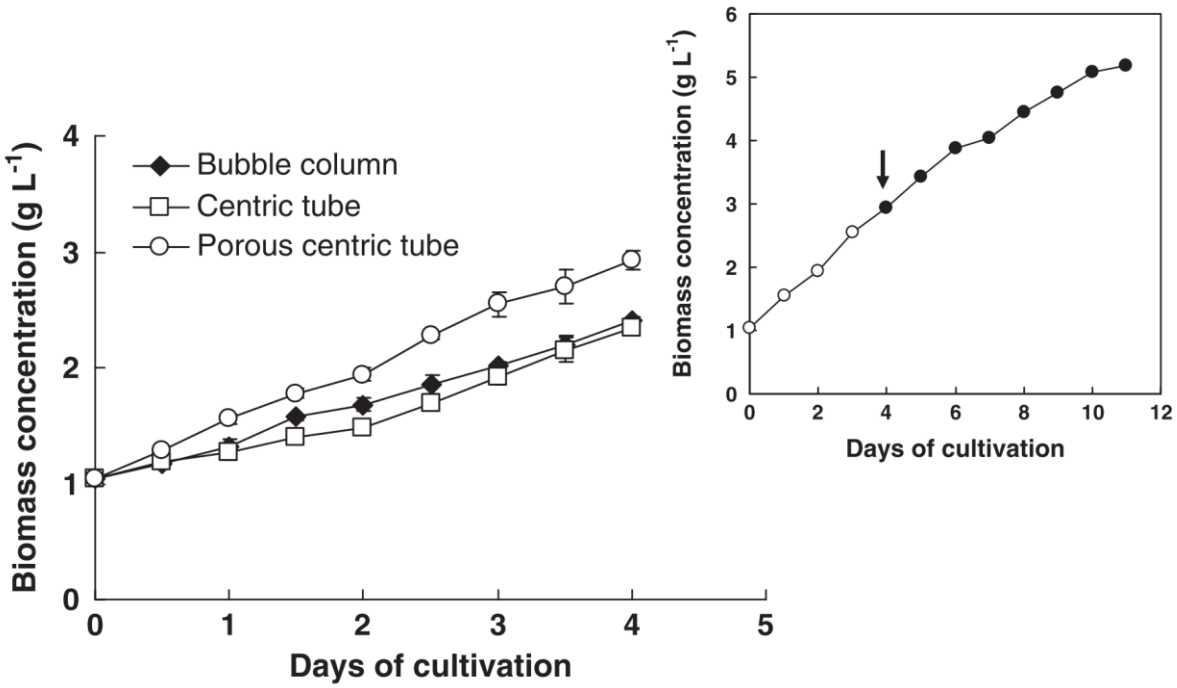

Figure 10. Growth curves of *Chlorella* sp. NCTU-2 cultured in the three different types of photobioreactor in batch and fed-batch culture mode. The cultures were aerated with 5% CO_2, which was adjusted by an individual gas flow meter. The initial cell density for each culture was approximate 1 g L^{-1}. The arrow indicates the time when cultivation in the porous centric-tube photobioreactor was changed to fed-batch cultivation, in which the feed medium was supplemented with 750 mg $NaNO_3$ and 44.11 mg $NaH_2PO_4 \cdot H_2O$ per liter every 2 d.

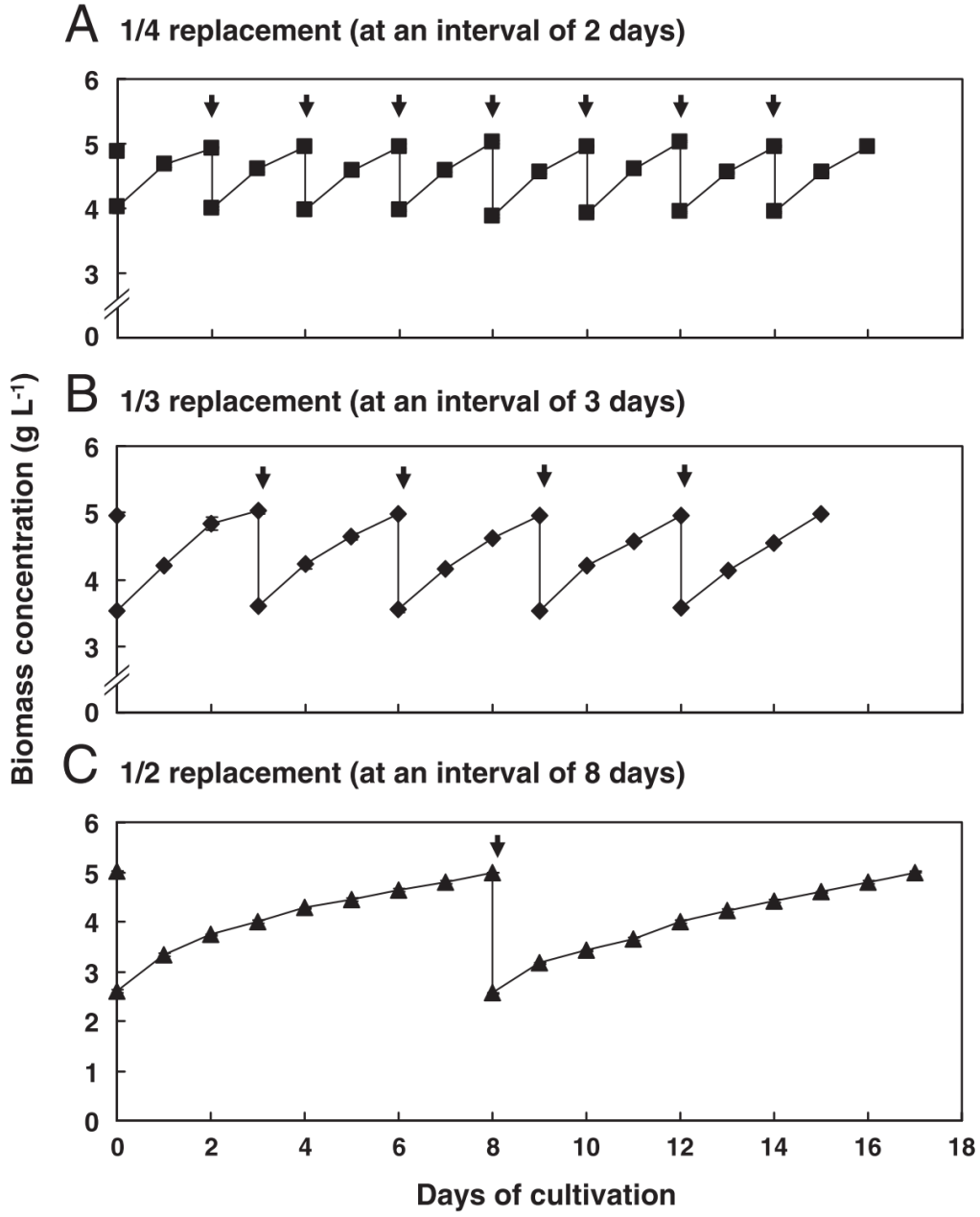

Figure 11. Growth profiles of *Chlorella* sp. NCTU-2 cultured in semicontinuous culture mode with 5% CO_2 aeration and operated by 1/4, 1/3 and 1/2 replacements in the porous centric-tube photobioreactor. The microalgal cells were pre-cultured in a fed-batch culture until they reached approximately 5 g L^{-1}. After the pre-culture, the culture medium was replaced with volume ratios of one half (1/2 replacement; the cultured broth was replaced at intervals of 2 days), one third (1/3; 3 days), and one fourth (1/4 replacement; 8 days) of fresh medium. The cultures were continuously operated for about 18 days. The arrows indicate the times when cultured broth was removed and fresh medium was added.

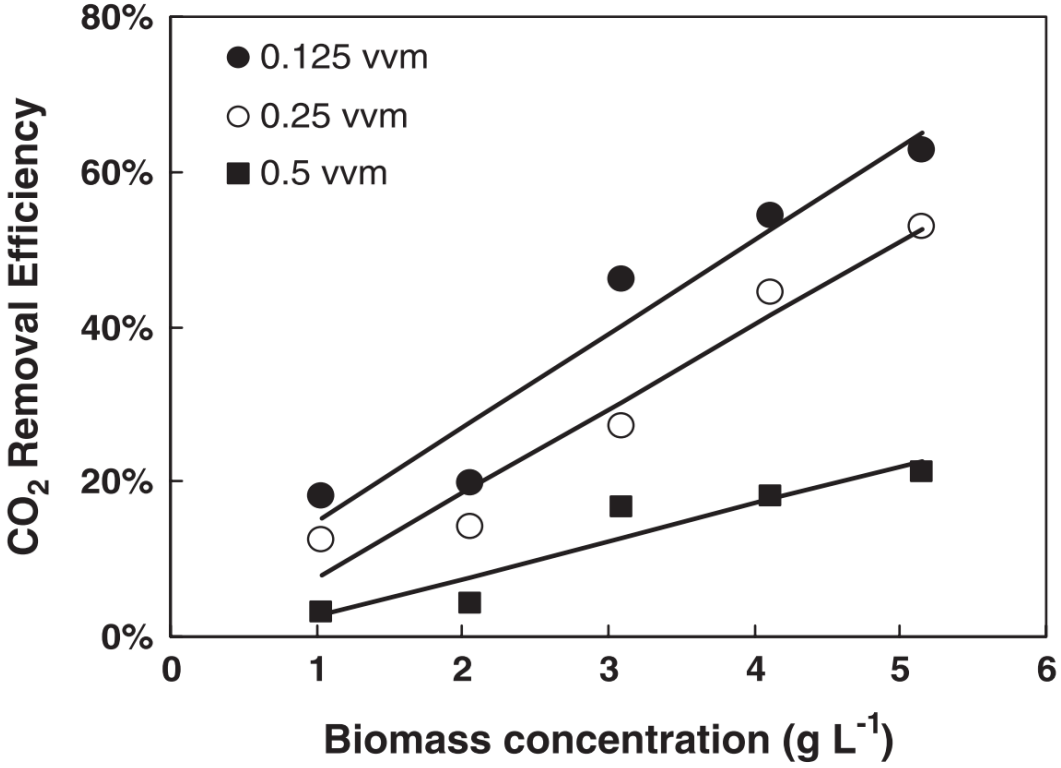

Figure 12. CO_2 removal efficiency in *Chlorella* sp. NCTU-2 cultures operated at different aeration rates and biomass concentrations. The microalgal cultures were pre-cultured in a fed-batch culture. Then, different densities of cultured microalgal cells were obtained by centrifugation. The microalgal cells were resuspended in fresh medium to achieve biomass concentrations of 1.03, 2.06, 3.09, 4.12, and 5.15 g L^{-1}. The cultures were operated in the porous centric-tube photobioreactor and aerated with 10% CO_2 at different gas flow rates of 0.125, 0.25, and 0.5 vvm. The CO_2 removal efficiency was determined by measuring the CO_2 concentration difference between the influent load and the effluent load, which were monitored by CO_2 sensors.

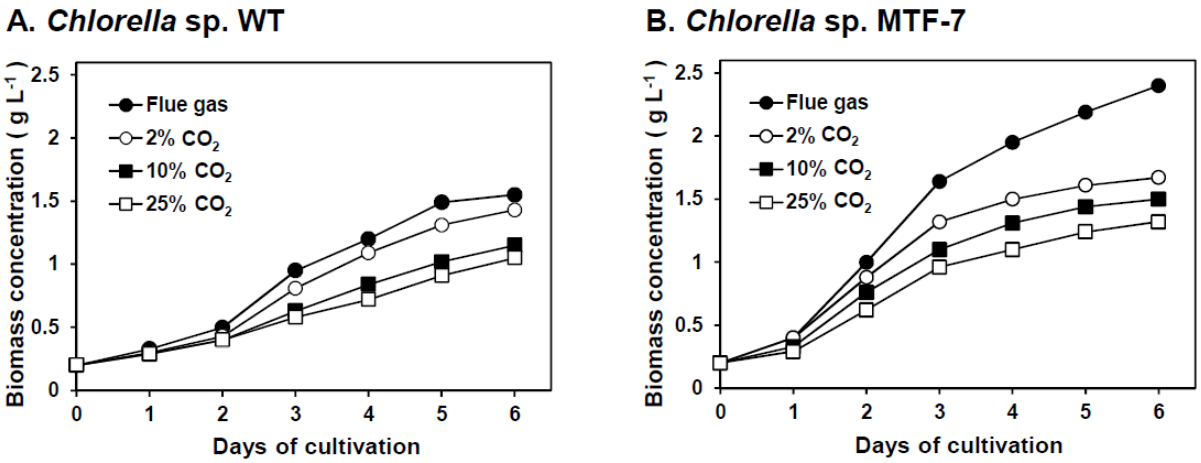

Figure 13. Growth profiles of *Chlorella* sp. (wild-type, WT) (**A**) and its mutant, *Chlorella* sp. MTF-7 (**B**), cultured in an indoor photobioreactor aerated with continuous flue gas or CO_2-enriched gas (2, 10, or 25%). The initial biomass concentration was approximately 0.2 g L^{-1}. The microalgal 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.

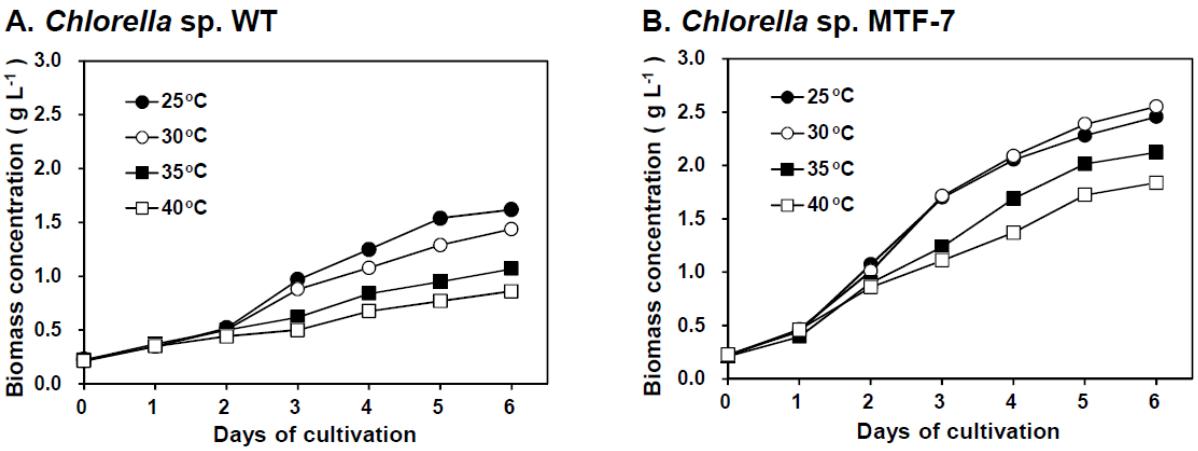

Figure 14. Growth profiles of *Chlorella* sp. (wild-type, WT) (**A**) and its mutant, *Chlorella* sp. MTF-7 (**B**), cultured in an indoor photobioreactor aerated with continuous flue gas and operated at different temperatures (25, 30, 35 or 40°C). The initial biomass concentration was approximately 0.2 g L^{-1}. The microalgal cells were cultivated at ~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.

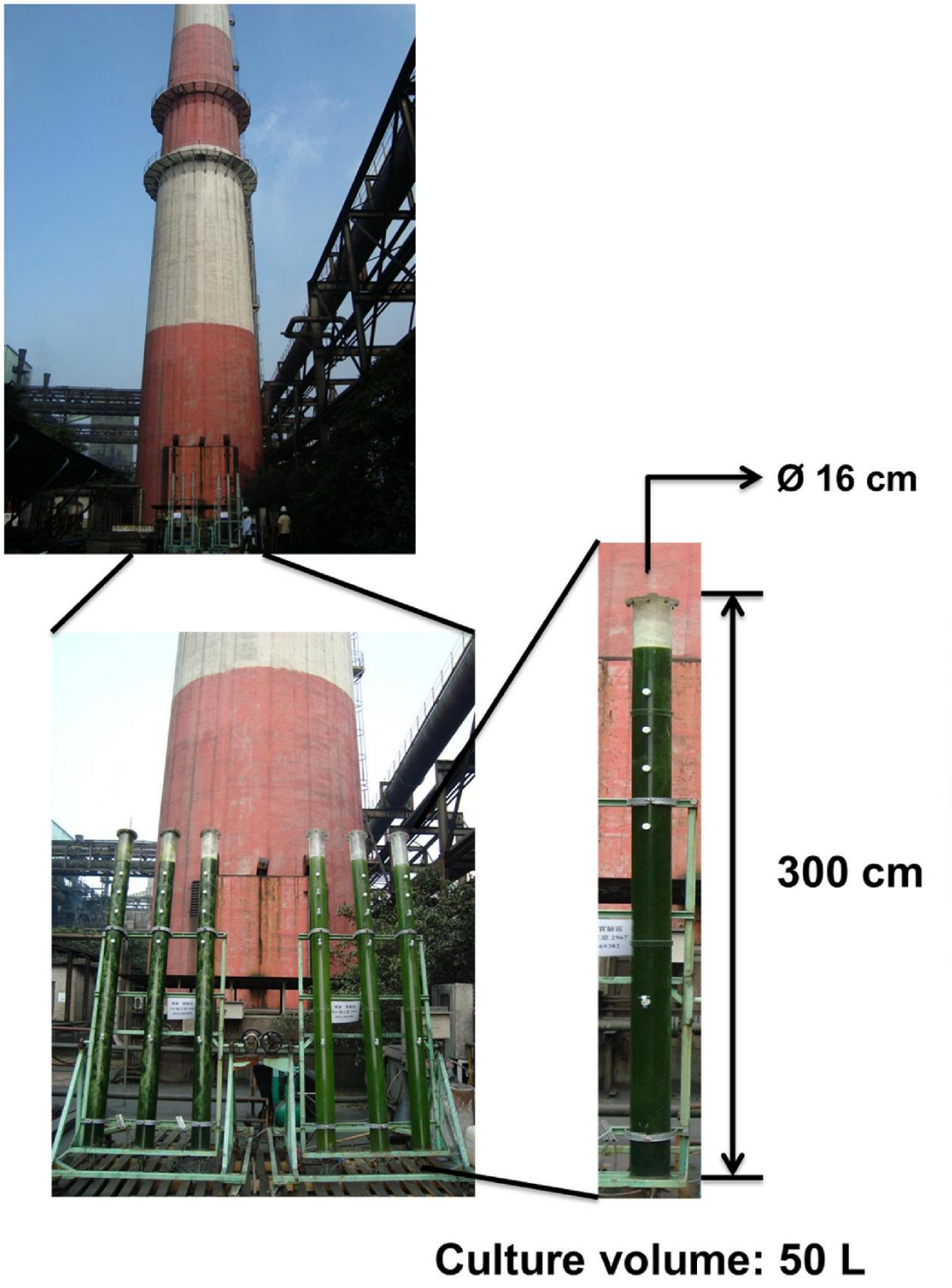

Figure 15. The outdoor photobioreactor culture system next to the smokestack of a coke oven at the China Steel Corporation, Kaohsiung, Taiwan. The outdoor photobioreactors are cylindrical and made of acrylic polymer. The column is 300 cm in length and 16 cm in diameter. Working volume of the photobioreactor is 50 L.

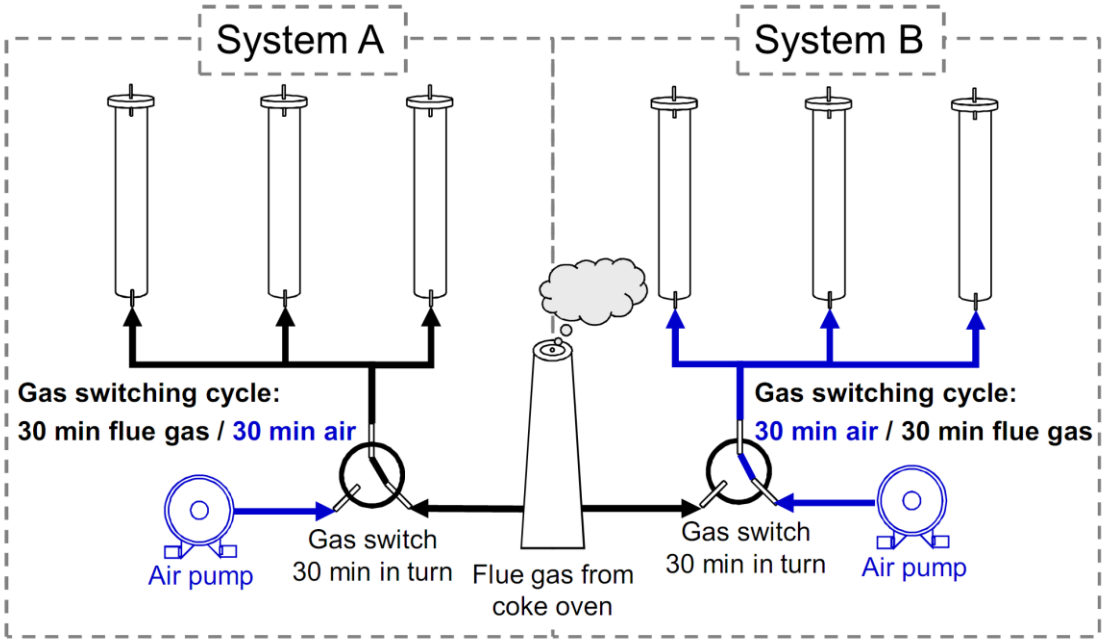

Figure 16. A double-set of photobioreactor system for flue gas-switching cycle operation used in this study. System A and system B were used for the flue gas- switching cycle operation (30 min flue gas/30 min air aeration in one system and 30 min air/30 min flue gas aeration in the other system). This double-set of photobioreactor system was alternately aerated with flue gas to achieve constant CO_2 capture from the flue gas.

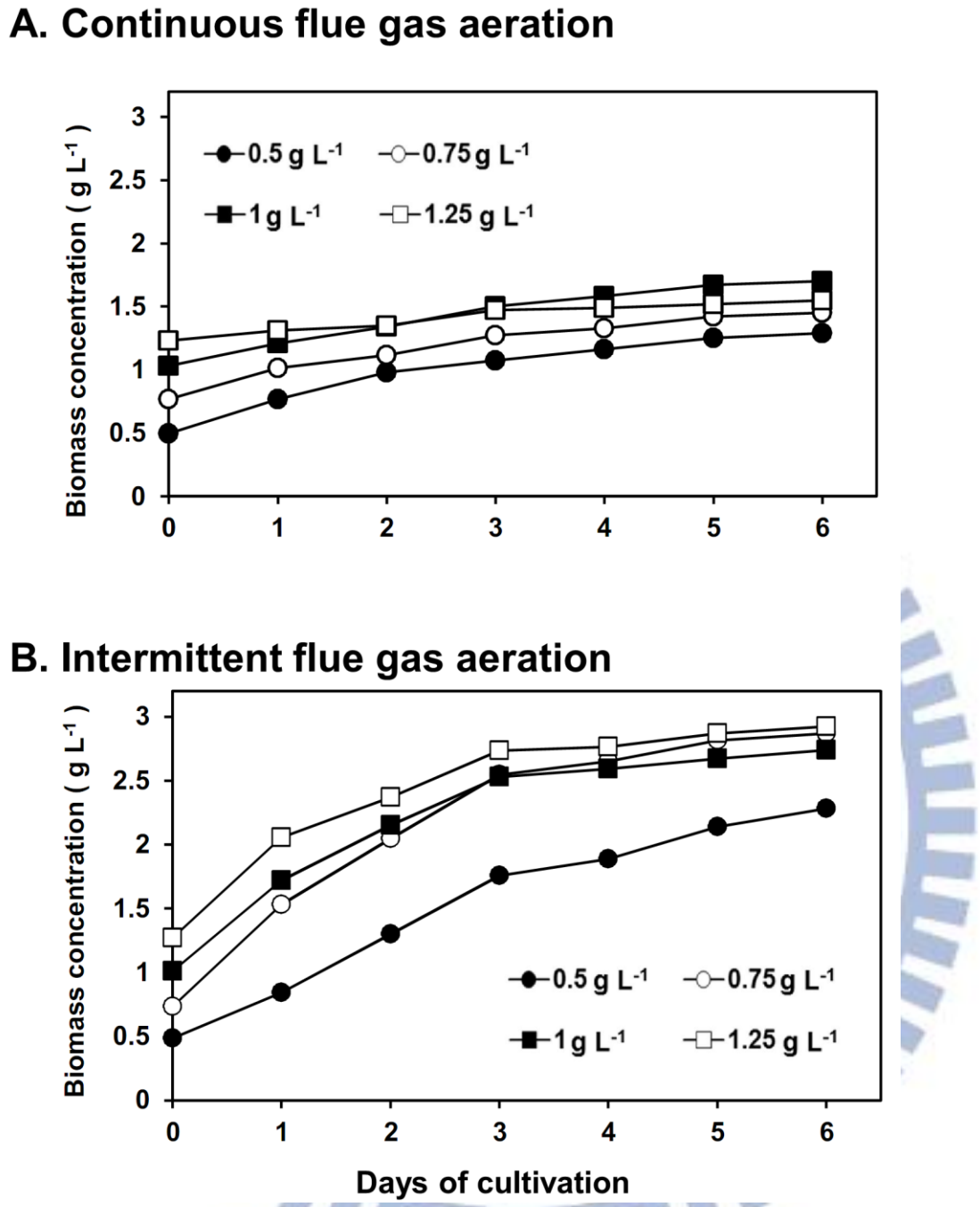

Figure 17. Growth profiles of *Chlorella* sp. MTF-7 cultured in an outdoor photobioreactor aerated with continuous (**A**) or intermittent flue gas (**B**). The initial biomass concentrations were 0.5, 0.75, 1.0 and 1.25 g L^{-1}. The microalgal cells were cultivated during the day (1,000-1,800 μmol 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.

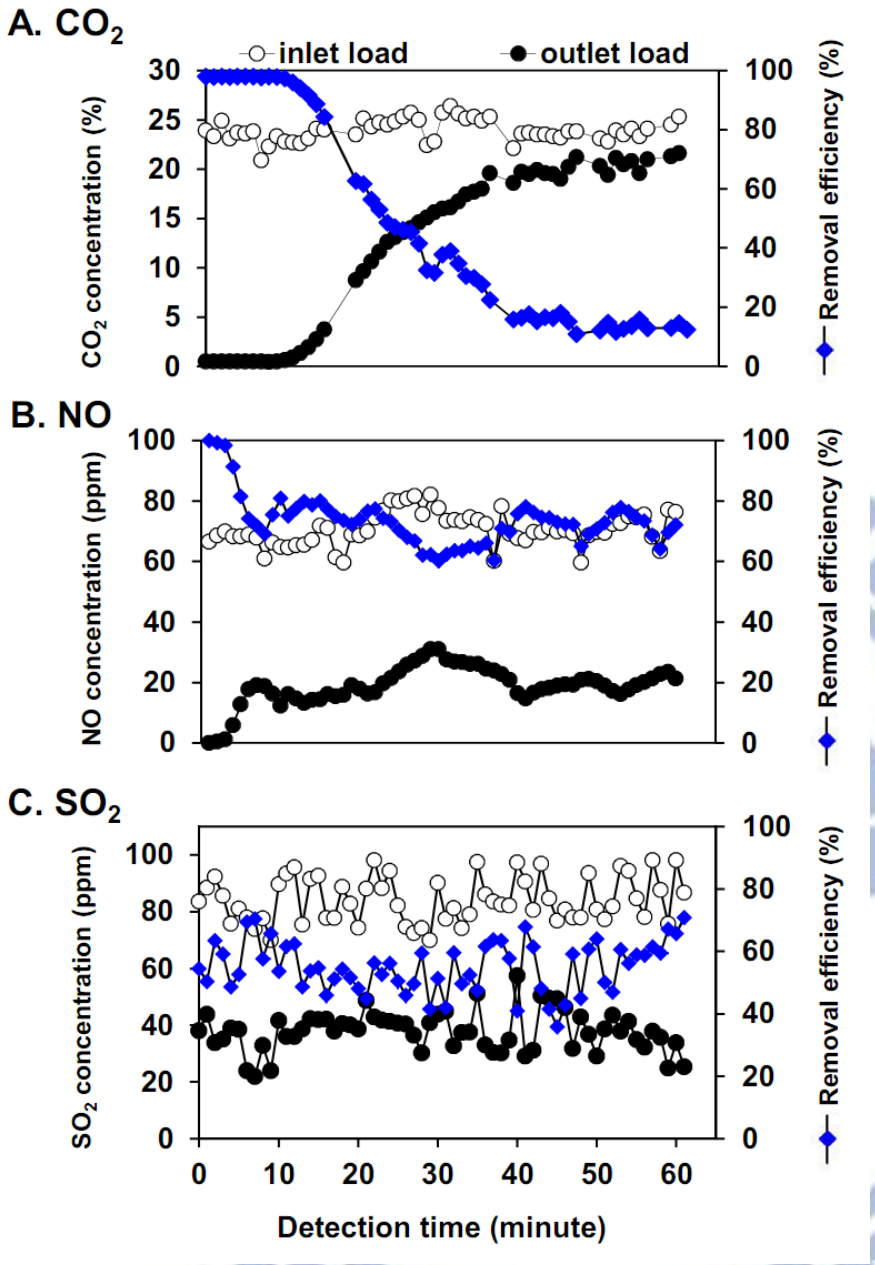

Figure 18. The efficiency of CO_2 (**A**), NO (**B**) and SO_2 (**C**) removal from flue gas by *Chlorella* sp. MTF-7 cultures under continuous flue gas aeration. The biomass concentration of the microalgal culture was approximately 2 g L^{-1}, and it was cultivated during the day (1,000-1,800 μmol m^{-2} s^{-1}). The flue gas was provided under continuous operation at 0.05 vvm. The CO_2, NO and SO_2 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 CO_2, NO and SO_2 removal efficiency (blue diamond) was calculated from the difference in the CO_2, NO and SO_2 concentrations of the inlet and outlet loads.

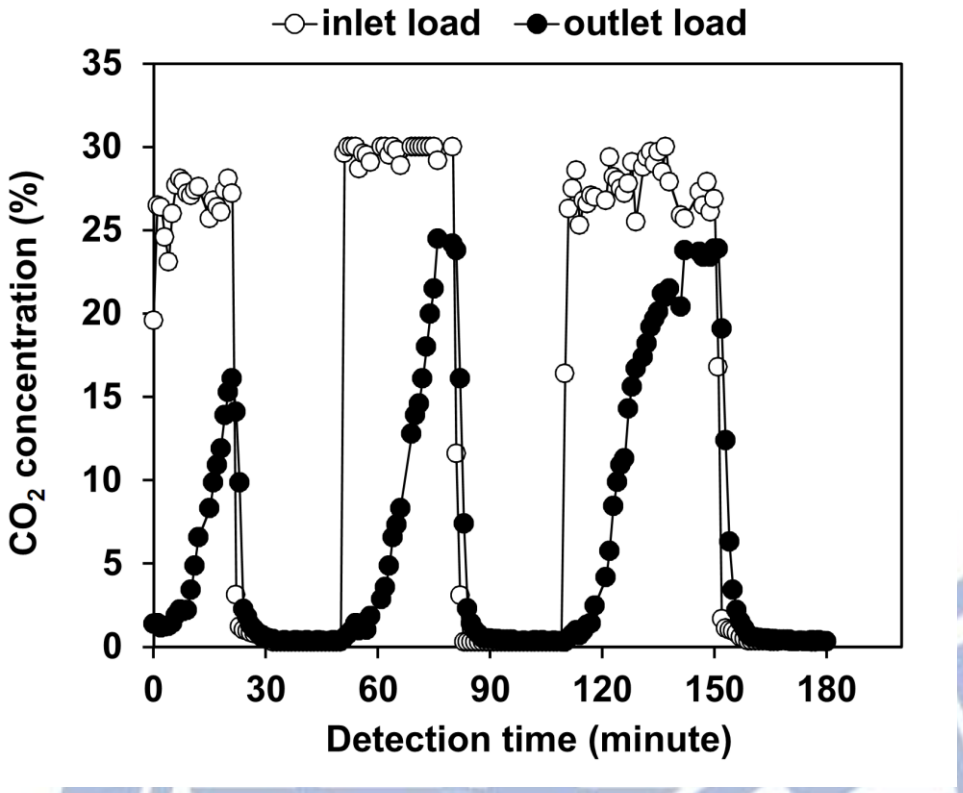

Figure 19. The patterns of the inlet and outlet load CO_2 concentrations of *Chlorella* sp. MTF-7 cultures aerated with intermittent flue gas at 0.05 vvm at different time intervals. The flue gas was controlled by a gas-switching cycle operation. A gas-switching cycle was performed with flue gas inlet loads for 20, 30 or 40 min, followed by air aeration for 30 min after the flue gas aeration. The CO_2 concentration was monitored once every minute at the inlet load (open circle) and the outlet load (solid circle) with a flue gas analyzer.

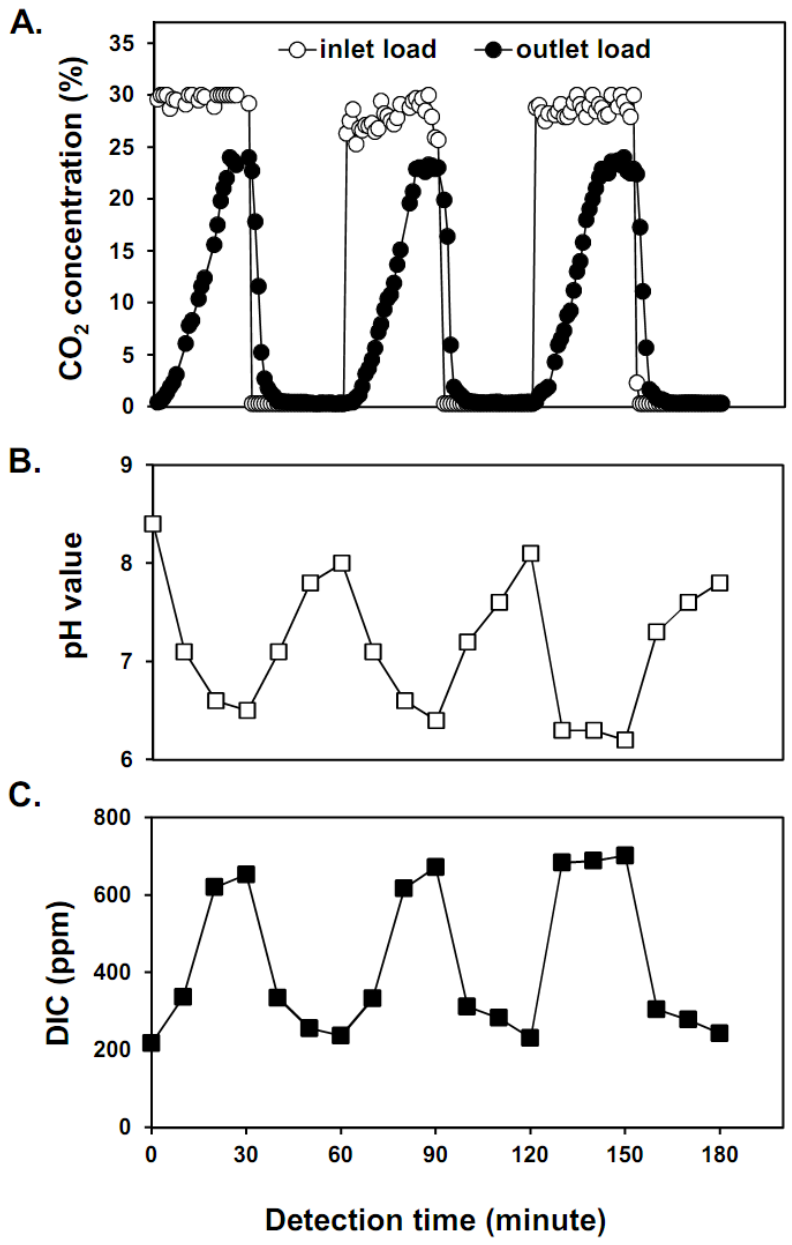

Figure 20. The patterns of the inlet and outlet load CO_2 concentrations (**A**), pH value (**B**), and dissolved inorganic carbon (DIC) concentrations (**C**) of *Chlorella* sp. MTF-7 cultures aerated with intermittent flue gas at 0.05 vvm. The flue gas was controlled by a gas-switching cycle operation. 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 CO_2 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.

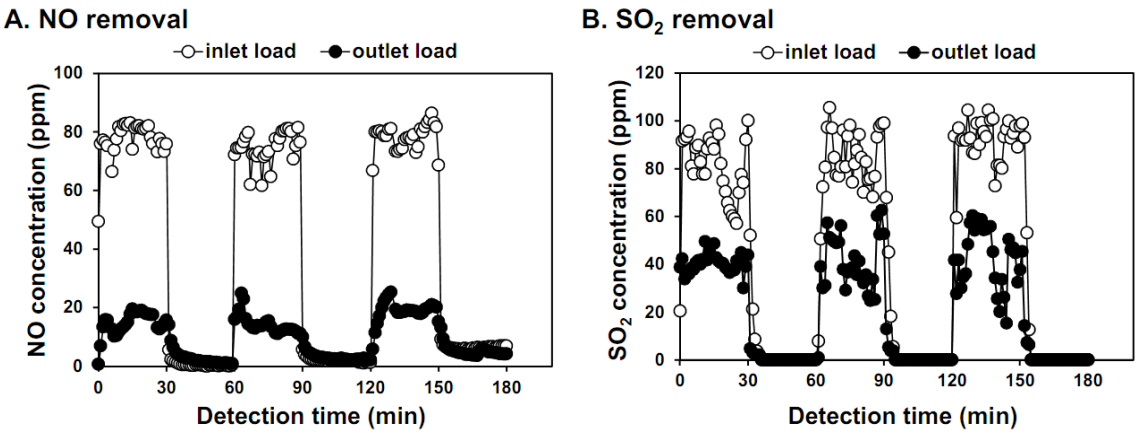

Figure 21. The patterns of the inlet and outlet load NO (**A**) and SO_2 (**B**) concentrations of *Chlorella* sp. MTF-7 cultures aerated with intermittent flue gas at 0.05 vvm. The NO and SO_2 concentrations were monitored in real time at the inlet load (open circle) and the outlet load (solid circle) gas with a flue gas analyzer once every minute.