國 立 交 通 大 學

生 物 科 技 學 院

生 化 工 程 研 究 所

碩 士 論 文

建立一光生物反應系統用於微藻的高密度養殖與二氧化碳的減量

Establishing a Photobioreactor System in the High-Density Microalgal Culture and Carbon Dioxide Reduction

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中 華 民 國 九 十 八 年 七 月

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國立交通大學 生化工程研究所碩士班

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建立一光生物反應系統用於微藻的高密度養殖與二氧化碳的減量

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摘　　要

微藻為行光合作用之生物體，可利用太陽能將水與二氧化碳（CO_2）轉換成生物質能（biomass），由於全球暖化日益嚴重，因此利用微藻減量環境中的二氧化碳並生產生物質是最具有潛力的方法之一。本研究所採用的五種微藻分別為 *Chlorella* sp.、*Nannochloropsis oculata*、*Skeletonema costatum*、*Isochrysis aff. galbana* 及 *Tetraselmis chui*，將此五種微藻於相同的環境下培養，並在 f/2 培養液與供給空氣的情況下，*Chlorella* sp.，*Nannochloropsis oculata*，*Skeletonema costatum*，*Isochrysis aff. galbana* 及 *Tetraselmis chui* 的生長率分別為 1.55、1.51、0.5、0.72 及 0.99 d^{-1}，並且從中篩選出 *Chlorella* sp.及 *Nannochloropsis oculata* 進行 CO_2 減量並生產生物質之研究。在本研究中，我們將 *Chlorella* sp.和 *Nannochloropsis oculata* 培養於封閉式光生物反應器中，經由半連續式的培養技術達到 CO_2 減量與高密度生產生物質的目標。首先，我們測量細胞密度和 CO_2 通入之濃度對微藻的影響，當微藻培養於 10 及 15% CO_2 下，生長會被抑制，但可經由高濃度微藻的接種（細胞密度 $>9\times10^7$ cells/mL）以及藻體事先培養於 2% CO_2 濃度下可改善抑制的情況發生，再將其轉入半連續式光合生物反應系統，通入 2、5、10 和 15% 的 CO_2 濃度下持續培養八天，我們測得在不同 CO_2 濃度之間的生長曲線是非常相似的。

利用半連續式光合反應系統（800 mL）通入 2、5、10 和 15%的 CO_2 培養後，CO_2 減量成果分別為 0.261、0.316、0.466 和 0.573 g/hr，而 CO_2 的減量效率則分別為 58、27、20 和 16%，且即使細胞生長在通入高濃度 CO_2 的培養條件下，生物質產量有下降的趨勢，但仍然看的出在 15%CO_2 條件下生長的 *Chlorella* sp.之生物質的生產量是具有生產潛力的，在本研究中也建立並運作六個反應器並聯式系統，且此系統通入不同濃度的 CO_2 培養之 CO_2 移除效率與單一式光合生物反應系統是相似的。於六個反應器並聯式系

統中，其 CO_2 總減量與生物質的總生產量得約為單一式的六倍。再則，在本研究中亦採用半連續式培養系統研究 CO_2 的濃度對於 *Nannochloropsis oculata* 生產生物質之影響。在 2% CO_2 培養條件下，生物質之生產量 0.480 g/L/day 為最高，即使在 15% CO_2 培養條件下，生物質之生產量亦可達 0.372 g/L/day。

這些結果指出光合生物反應系統若應用於 CO_2 減量，可以藉由多組並聯的方式來針對大量的廢氣處理。而採用每天置換 1/2 的半連續式系統並通入 2% CO_2 培養，是對於 *Chlorella* sp.和 *Nannochloropsis oculata* 最適的生物質生產條件。

關鍵詞：微藻，小球藻，擬球藻，二氧化碳，光合生物反應器，生物質

Establishing a photobioreactor system in the high-density microalgal culture and carbon dioxide reduction

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Abstract

Microalgae as photosynthetic organisms can use solar energy to convert water and carbon dioxide (CO_2) into biomass. Facing the increasing concerns about global warming, the reduction of CO_2 emission to acceptable levels by utilizing microalgae to consume CO_2 and to produce biomass is a potential approach. Five microalgal strains, *Chlorella* sp., *Nannochloropsis oculata*, *Skeletonema costatum*, *Isochrysis aff. galbana* and *Tetraselmis chui*, were first used in this study. The growth potential of *Chlorella* sp., *Nannochloropsis oculata*, *Skeletonema costatum*, *Isochrysis aff. galbana* and *Tetraselmis chui* cultured in the f/2 medium (designed as normal cultural medium) and given air were 1.55, 1.51, 0.5, 0.72 and 0.99 d^{-1}, respectively. *Chlorella* sp. and *Nannochloropsis oculata* were selected for the studies of CO_2 reduction and biomass production. In this study, *Chlorella* sp. and *Nannochloropsis oculata*, were cultured in a closed system of photobioreactors in the semi-continuous cultivation conditions for the exploration of CO_2 reduction and high-density microalgal biomass production. First, we determined the effects of cell density and CO_2 concentration in airstreams on the growth of microalgae. The growth inhibition when the microalgal cells were cultured in 10 and 15% CO_2 aeration could be overcome via a high density of microalga inoculum (up to 9×10^7 cells/mL) and pre-adapted culture with 2% CO_2 aeration. The cultures were then transferred into a semi-continuous photobioreactor system aerated with 2, 5, 10 and 15% CO_2. The profiles of growth curve of microalgal cultures aerated with different CO_2 concentration were similar.

Amount of CO_2 reduction and CO_2 reduction efficiency of the *Chlorella* sp. cultures in the semi-continuous cultivation (800 mL) under 2, 5, 10 and 15% CO_2 aeration were 0.261, 0.316, 0.466 and 0.573 g/hr and 58, 27, 20 and 16%, respectively. The biomass production

of *Chlorella* sp. cultures could maintain in 15% CO_2 aeration although biomass productions showed a decreased trend when the cells exposed to the airstreams with rising CO_2 concentration increased. A six-parallel photobioreactor system was also performed in this study, and the CO_2 reduction efficiency in the system was similar to the single photobioreactor in different concentrations of CO_2 aeration. Performances, including total amount of CO_2 reduction and biomass production of the six-parallel photobioreactor system was also determined and the result were around 6 folds compared those in the matched single photobioreactor. And then, the effects of concentration of CO_2 aeration on the biomass production and lipid accumulation of *Nannochloropsis oculata* in a semi-continuous culture were investigated in this study. The maximal biomass productivity in the semi-continuous system was 0.480 g/L/d with 2% CO_2 aeration. Even the *Nannochloropsis oculata* cultured in the semi-continuous system aerated with 15% CO_2, the biomass productivity could reach to 0.372 g/L/d.

These results indicate that the CO_2 reduction by microalgae incorporated photobioreactor could be extended to multiple parallel units of the photobioreactor for a large amount of waste gas treatment. To optimize the condition for long-term biomass yield from *Chlorella* sp. and *Nannochloropsis oculata*, these microalgae were suggested growing in the semi-continuous system aerated with 2% CO_2 and operating by one-day replacement.

Key words: Microalgae, *Chlorella* sp., *Nannochloropsis oculata*, Carbon dioxide, Photobioreactor, Biomass

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I. Research Background and Significance

1.1. Greenhouse effect and global warming

1.1.1. Global warming

Global warming induced by increasing concentrations of greenhouse gases in the atmosphere is of great concern. 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.

1.1.2. Greenhouse gases

Carbon dioxide (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 concentration, for example by the use of renewable energy sources or by terrestrial sequestration of carbon [IPCC, 2001]. 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].

1.1.3. Carbon dioxide removal from waste gas by different method

CO_2 capture from power plants entails the integration of a capture technology into a power plant system. The primary CO_2 capture technologies being considered are cryogenics, Adsorption, chemical absorption, and biological remediation.

Cryogenics

Cryogenics is refrigeration of the gas stream to reduce the vapor pressure so phase change occurs and the liquid CO_2 can be distilled out of the mixture. Significant energy is

required to cool the gas especially since the majority of power plant processes occur at high temperature. Without substantial new system integration, cryogenics does not appear either efficient or economically feasible for power plants [Kohl. 1997].

Adsorption

Adsorption, occurs by passing the flue gas stream through a microporous solid adsorbent stream so that surface forces capture the CO_2 on the surface of the adsorbent without chemical reaction. Modifications of this process include pressure swing adsorption and temperature swing adsorption, which rely on high pressure and temperature respectively to activate surface forces and then to low pressure or temperature to regenerate the adsorbent [ESRU. 2006]. Significant process and system development work is underway to implement absorption in power plants for CO_2 capture.

Chemical absorption

Chemical absorption entails passing the flue gas stream through an absorbent stream but in this case the CO_2 chemically reacts with the absorbent to reduce the Gibbs free energy of the mixture. The absorption reaction requires a low temperature of approximately 50^{o}C and a desorption reaction to regenerate the absorbent occuring at approximately 120^{o}C [ESRU. 2006]. Chemical absorption is most effective with low CO_2 concentrations and is therefore appropriate for flue gas processing where the CO_2 is diluted with air and steam. **Table 1-1** shows the chemical method of carbon dioxide reduction.

Biological remediation

Biological remediation harnesses the natural process that plants undergo to consume CO_2 and convert it into biological material. Photosynthesis is the most common method of biological remediation method, but some algae are known to utilize CO_2 in the absence of light. A portion of CO_2 in the atmosphere is absorbed biologically by terrestrial plant life. However given the increased CO_2 atmospheric concentration of 0.4 percent per year, the remediation rate does not keep pace with emissions. To increase the rate of biological remediation, bioreactors are being developed to integrate into power plant systems [Bayless. 2003].

1.2. Microalgae

1.2.1. Why microalgae

Microalgae are some of the most robust organisms on earth, able to grow in a wide range of conditions. The algae comprise one of the most diverse plant groups. And they grow in almost every habitat in every part of the world. A species range of 40 000 to 10 million has been estimated, with the majority being the microalgae [Hawksworth and Mound, 1991; Metting, 1996]. Microalgae are a highly diverse group of unicellular organisms comprising the eukaryotic protists and the prokaryotic cyanobacteria or blue-green algae. A diverse group of photosynthetic organisms, the algae have successfully adapted their metabolism to occupy different habitat extremes ranging from the polar regions to tropical coral reefs. The ability to withstand environmental stress is matched by the capacity of algae to produce a vast array of secondary metabolites, which are of considerable value in biotechnology programs including the aquaculture, health, and food industries [Andersen, 1996].

Microalgae are one of the earth's most important natural resources. They contribute to approximately 50% of global photosynthetic activity [Wiessner et al., 1995] and form the basis of the food chain for over 70% of the world's biomass [Andersen, 1996]. In recent years, the bio-regenerative 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 reduction, there is no requirement for further disposal of recovered CO_2 [Lee and Lee, 2003; Suh and Lee, 2003; Carvalho et al., 2006; 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 sea water which solubilizes high amount of CO_2. 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. The CO_2 reduction by microalgal photosynthesis and biomass conversion into health food, food additives, feed supplements, and biofuel is considered a simple and appropriate process for CO_2 circulation on Earth [Takagi et al., 2000].

1.2.2. Environment factor affect algae growth

Like plants, algae use the sunlight for the process of photosynthesis. Photosynthesis is an important biochemical process in which plants, algae, and some bacteria convert the energy of sunlight to chemical energy. Algae capture light energy through photosynthesis and convert inorganic substances into simple sugars using the captured energy.

The most important parameters regulating algal growth are nutrient quantity and quality, light, pH, turbulence, salinity and temperature. The most optimal parameters as well as the tolerated ranges are species specific. Also, the various factors may be interdependent and a parameter that is optimal for one set of conditions is not necessarily optimal for another.

When cultivating algae, several factors must be considered, and different algae have different requirements. Essential factors include water, carbon dioxide, minerals and light would affect microalgal growth.

Temperature

Temperature influences respiration and photorespiration more strongly than photosynthesis. When CO_2 or light is limiting for photosynthesis, the influence of temperature is insignificant. With an increase in temperature, respiration will rise significantly, but the flux through the Calvin cycle increases only marginally. Thus, the net efficiency of photosynthesis declines at high temperatures. This effect can worsen in suspension cultures by the difference in decrease of CO_2 and O_2 solubility at elevated temperatures. The water must be in a temperature range that will support the specific algal species being grown. Optimal culture temperature vary with the species and strains. The optimal Temperature for phytoplankton cultures is generally between 20 and 30^{o}C. Temperatures lower than 16^{o}C slow down growth; temperatures higher than 35^{o}C are lethal for a number of species.

Light energy

Light as the energy source for photoautotrophic life is the principal limiting factor in photobiotechnology [Kirk, 1994]. At illumination intensities above the light compensation point the rate of photosynthesis is directly proportional to light intensity, until at high illumination intensities damage to the photosynthetic receptor system occurs within a few minutes (photoinhibition). In most microalgae, photosynthesis is saturated at about 30% of the total terrestrial solar radiation, i.e. 1,700–2,000 μmol/m^2/s. Some picoplankton species

grow with optimal rates at 50 μmol/m^2/s and are photoinhibited at 130 μmol/m^2/s. Phanerophytes, like most agricultural crops with light limitation values of 900 μmol/m^2/s, are clearly adapted to higher PFD than microalgae are. Stirred fermenters illuminated with various submersed luminous elements or light pipes facilitate an average productivity in the range of 100–1,000 mg DW/day. This appears to be the upper limit at a surface to volume ratio of 2–8 m^2/m^3 typical for this illumination design. Laboratory bioreactors based on this principle are very well suited to physiological and autecological studies as well as for the establishment and testing of miniature ecosystems, but they cannot be used for scaling up. In tubular or plate-type photobioreactors, surface to volume ratios of 20–80 m^2/m^3 and light incidence values (PAR) up to 1,150 μmol/m^2/s are achieved. At a layer thickness of up to 5 mm, a productivity of 2–5 g DW/day can be achieved [Chini Zitelli et al., 2000]. Despite growing interest in recent years, there are only a few references in the literature regarding the short-term processes of photoadaptation, on light inhibition or saturation effects in closed photobioreactors. Photoadaptation requires at least 10–40 min, which can explain the discrepancy between the productivity of open-air algal cultures and their light optimum. Photoinhibitory processes are time-dependent; however, in this case irreversible destruction is supposed to occur even after only a few minutes of light stress, exceeding 50% damage after 10–20 min.

Light must not be too strong nor too weak. In most algal-cultivation systems, light only penetrates the top 3 inches (7.6 cm) to 4 inches (10 cm) of the water. This is because as the algae grow and multiply, they become so dense that they block light from reaching deeper into the pond or tank. Algae only need about 1/10 the amount of light they receive from direct sunlight. Direct sunlight is often too strong for algae.

In order to have ponds that are deeper than 4 inches algae growers use various methods to agitate the water in their ponds. Paddle wheels can be used to circulate (stir) the water in a pond. Compressed air can be introduced into the bottom of a pond or tank to agitate the water, bringing algae from the lower levels up with it as it makes its way to the surface.

Apart from agitation, another means of supplying light to algae is to place the light in the system. Glow plates are sheets of plastic or glass that can be submerged into a tank, providing light directly to the algae at the right concentration.

Mixing

Mixing of microbial cultures is important for homogeneous distribution of cells, metabolites, and heat, and for transfer of gasses across gas–liquid interfaces. In microalgal cultures, mixing also affects the light regimen [Richmond, 2000; Grobbelaar, 2000]. Fluctuations in light intensity faster than 1 sec enhance specific growth rates and productivities of microalgal cultures [Nedbal et al., 1996; Ogbonna and Tanaka, 2000; Janssen et al., 2001; Yoshimoto et al., 2005]. In outdoor cultures exposed to photosynthetic photon flux densities above 1,000 μmol/m^2/s light exposure times should be as short as 10 msec to maintain high photosynthetic efficiency [Janssen et al., 2001].

CO_2

The CO_2 reduction rate is related directly to light utilization efficiency and to cell density of microalgae. Microalgal CO_2 reduction 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 [Costa et al., 2004; Chen et al., 2006; 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, 2007b; Keffer and Kleinheinz, 2002]. 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.

Nutrients, salinity, and pH-value

A sufficient nutrient supply for microalgae is a precondition for optimal photosynthesis. Nutrients must be controlled, so algae will not be starved and nutrients will not be waste. Some types of culture method could modulate the composition of nutrients to control the capability of some algae to take up and to metabolize fixed carbon, i.e., to grow heterotrophically. Although this restricts the range of algae that may be grown, the system has been successfully used to produce α-tocopherol [Ogbonna et al., 1998], ascorbic acid [Running et al., 1994], aquaculture organisms [Day and Tsavalos, 1996], fatty acids [Barclay et al., 1994], and leutin [Shi et al., 1997]. Deviations from optimum pH, osmotical conditions and salinity will cause physiological reactions and productivity problems.

Therefore, these easily controllable conditions should be maintained in optimum ratios in

photobioreactors. Organic carbon sources seem to be important for some mixotrophic or even pure heterotrophic microalgal biomass production systems. Therefore, organic wastes as well as pure simple organic substances like acetic acid or various sugars could be investigated for their possible use in microalgal production.

1.2.3. Composition of algae

All microalgae primary comprise of the following, in varying proportions: proteins, carbohydrates, fats and nucleic acids. The percentages vary with the types of microalgae. (**Table 1-2**)

1.2.4. Applications of microalgae

Microalgae produced in large-scale commercial systems are used for the most part as the whole biomass. There has also been an upsurge in research and development on the utilization of microalgae as sources of a wide range of metabolites, such as bioactive compounds, pigments and oils. Dried biomass is generally utilized for health foods, food additives, feed supplements, and other uses. Live microalgae are usually served as larval diets in aquaculture. Current use of microalgal product in the world is summarized in **Table 1-3**.

Health foods

Microalgae health foods are available in the form of tablets, granules and drinks. This rapid spread may be due to the fact that various health-promoting effects of *Chlorella* have been clarified.

Yamagishi et al. [1962] reported that *Chlorella* showed therapeutic efficacy on gastric ulcer, clinical tests have been done actively on many kinds of disorders such as wounds [Hasuda and Mito, 1966], constipatio, leucopenia [Saito et al., 1966], anemia [Sonoda, 1972], hypertension [Miyakoshi et al., 1980], diabetes [Fukui, 1979], infant malnutrition [Tokuyasu, 1983] and neurosis [Sonoda and Okuda, 1978]. The validity may be attributable to composite effects not only of nutritive components such as vitamins, minerals, dietary fibers and proteins but of a preventive action against atherosclerosis and hypercholesterolemia by glycolipids and phospholipids [Sano and Tanaka, 1987] and antitumor actions by

glycoproteins, peptides, nucleotides and related compounds [Konishi et al., 1985; Tanaka et al., 1984].

Spirulina (Arthrospira) has a long history as food; it grows profusely in certain alkaline lakes in Mexico and Africa and has been eaten since time immemorial by inhabitants in the neighborhood. *Spirulina* has become the object of attention around the world because of the high content of protein and the excellent nutritive value. Spray-dried *Spirulina* powder is fabricated into tablets with or without added calcium or vitamin C, being marketed as health foods. Since modem science revealed that they are eaten mainly as a substitute for green vegetables, because the content of vitamins and minerals in *5* g dried *Spirulina* corresponds to 100 g vegetables [Kato, 1991].

Dunaliella is well known to accumulate *β*-carotene to more than 10% dry weight under appropriate growth conditions. Beta-carotene was attracted the attention of many people in Japan because of the anticarcinogenic activity of carotenoids, especially *β*-carotene, in foodstuffs [Nishino, 1993]. Dried biomass of *Dunaliella* is imported from Australia and Israel, and its capsules and tablets are placed on the market as a health food [Yamaguchi, 1992].

Food additives

The first addition of *Chlorella* to foods was the production of fermented milk by utilizing a stimulating effect of a *Chlorella* extract on the growth of *Lactobacillus* [Mitsuda et al., 1961]. Nowadays dried biomass or extracts of *Chlorella* is used as additives to fermented soybeans (ex: natto), vinegar and liquors on account of the effects on growth of microorganisms. Or it is used as additives to drinks, green tea, *tofu* (bean curds), liquors, candies, bread, noodles, etc. because of the taste- and flavor-adjusting actions. Furthermore, dried *Chlorella* is added to noodles and Western and Japanese cakes as a coloring agent [Maruyama and Ando, 1992].

Spirulina contains high levels of the blue biliprotein, phycocyanin [Kageyama et al., 1994]. A blue food pigmenter manufactured from phycocyanin is marketed under the commercial name of “Lina blue A” [Kato, 1985]. It is a blue powder readily soluble in water. It is used as a natural food color in ice cream, chewing gum, jelly, candy, yogurt, 'wasabi' paste, etc. [Kato, 1991].

Feed supplements

Dried biomass or extracts of *Chlorella* are added to diets to improve the quality of cultured fish; for instance, it has been reported that enhancement of resistibility to diseases [Nakagawa et al., 1981] and improvement of the flesh in quality [Nakagawa et al., 1984] were attained by adding *Chlorella* extract at approximately 1% to the diet of the ayu (sweet smelt) *Plecoglossus altivelis.* Certain strains of *Chlorella* become red or orange in a nitrogen-limited and/or hypersaline medium, accumulating a large quantity of a red carotenoid and astaxanthin. Such a biomass is considered to be an effective feed supplement for pigmentation of cultured fish and shellfish [Sano, 1993].

Over 50% of the total production of *Spirulina is* actually used as feed supplements, though it is generally supposed that the most part is consumed as health foods [Kato, 1992]. *Spirulina* is rich in carotenoids, especially zeaxanthin and *β*-carotene and can exert pigmentation effects when supplemented to diets for cultured fish and shellfish such as striped jack [Okada et al., 1991], kuruma prawn [Kato, 1992], and black tiger prawn [Liao et al., 1993]. It has also been reported that the supplementation of *Spirulina* to a hen feed improved the yellow color of egg yolk. Feeding the diets supplemented with *Spirulina* to cultured yellowtail, masu salmon and eel was reported to have brought about such health-promoting effects such as decreasing in mortality and increasing in growth [Kato, 1992]. In addition, an improving effect on the flesh quality was observed for cultured red sea bream [Yamaguchi et al., 1987] and striped jack [Liao et al., 1990; Watanabe et al., 1990]. This is largely due to a reduction of lipid content in the muscle that may be caused by *γ*-linolenic acid, which is specifically rich in *Spirulina* [Liao, 1990].

Nannochloropsis and *Chlorella* are applied in the production of zooplankton such as the rotifer *Brachionusplicatilis* and the copepod *Tigriopusjaponicus,* both of which are important larval and juvenile feeds of fish [Fukusho, 1981; Kitajima, 1983; Yoneda, 1983]. It has been shown that vitamin B_{12} is necessary for the growth of the rotifer [Scott, 1981] and that n-3 highly unsaturated fatty acids (n-3 HUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) synthesized by some microalgae, for instance, *Nannochloropsis oculata* and *P. tricornutum* are essential for the growth and survival of marine fish [Kitajima, 1983]. Therefore, vitamin B_{12} and/or n-3 HUFA-fortified *Chlorella* products and various types of *Nannochloropsis* products are in market [Hirayama et al., 1989; Maruyama et al., 1989, 1990; Okauchi, 1992]. Recently, a simple two-step culture using *Chlorella regularis* and *I. galbana* was reported for DHA enrichment of rotifers [Takeyama et al., 1996]. In this

context, it should be noted that the fatty acid composition as well as the proximate composition of microalgae vary considerably depending on culture conditions and growth phases [Okauchi et al., 1990; Tatsuzawa and Takizawa, 1995].

Biofuels

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].

1.3. Microalgae culture system

Algae can be produced using a wide variety of methods, ranging from closely-controlled laboratory methods to less predictable methods in outdoor tanks. The terminology used to describe the type of algal culture includes:

Batch, Continuous, and Semi-Continuous

These are the three basic types of Phytoplankton culture.

Batch culture: The batch culture consists of a single inoculation of cells into a container of fertilized seawater followed by a growing period of several days and finally harvesting when the algal population reaches its maximum or near-maximum density.

Continuous culture: The continuous culture method, (i.e. a culture in which a supply of fertilized seawater is continuously pumped into a growth chamber and the excess culture is simultaneously washed out), permits the maintenance of cultures very close to the maximum growth rate.

Semi-continuous culture: The semi-continuous technique prolongs the use of large tank

cultures by partial periodic harvesting followed immediately by topping up to the original volume and supplementing with nutrients to achieve the original level of enrichment.

Indoor/Outdoor

Indoor culture is mainly by photobioreactor which allows controlling over illumination, temperature, nutrient level, contamination with predators and other competing algae; whereas outdoor algal mostly of raceway pond systems makes it very difficult to grow specific algal cultures for extended periods.

Open/Closed

Open cultures such as uncovered ponds and tanks (indoors or outdoors) are more readily contaminated than closed culture vessels such as tubes, flasks, carboys, bags, etc. Closed cultures are usually ponds covered with green house or a photobioreactor.

1.3.1. Open culture system

Open ponds resemble most closely the natural milieu of microalgae. Open systems include the use of managed lakes which may be up to 300 hectares [Schlipalius, 1991], unstirred open ponds like the β-carotene production plant at Hutt Lagoon in Western Australia [Borowitzka, 1991], circular ponds, paddle-wheel raceways, and sloping cascades [Oswald, 1988; Becker, 1994] (**Figure 1-1**). Despite a certain variability in shape, the most common technical design for open pond systems are raceway cultivators driven by paddle wheels and usually operating at water depths of 15–20 cm (**Figure 1-2**). At these water depths, biomass concentrations of up to 1,000 mg/l and productivities of 60–100 mg/day, i.e., 10–25 g/m^2/day, is possible. Similar in design are the circular ponds which are common in Asia and the Ukraine [Becker 1994].

All of these generally have the advantage of being relatively cheap to construct. However, problems associated with excessive shear forces damaging the algal ceils and the need for environmental control including pH, temperature, nutrient levels, osmotic potential, contamination by other algae, and grazing have restricted their use. Significant evaporative losses, the diffusion of CO_2 to the atmosphere as well as the permanent threat of contamination and pollution, are the major drawbacks of open pond systems. Also, the large area required must not be underestimated. The main disadvantage of this principle in terms of productivity seems to be the light limitation in the high layer thickness. Technically it is

possible to enhance light supply by reducing the layer thickness to a few centimeters or even millimeters, using thin layer inclined types of culture systems. Another problem of open systems is the maintenance of the desired microalgal population, which is possible only for extremophilic species and even there some contamination risks remain.

Open systems were the most important design principle for microalgal production [Richmond, 1990]. However, the preparation of high-value products from microalgae for applications in pharmacy and cosmetics appears to be feasible only on the basis of closed photobioreactors with the ability to reproduce production conditions and to be GMP-relevant (GMP: good manufacturing practice following ISO and EC guidelines).

1.3.2. Closed system

Closed photobioreactor are characterized by the regulation and control of nearly all the biotechnologically important parameters as well as by the following fundamental benefits [Pulz, 1992]: a reduced contamination risk, no CO_2 losses, reproducible cultivation conditions, controllable hydrodynamics and temperature, and flexible technical design.

The scale-up of simple closed container-based systems (tanks, hanging plastic bags) as a first generation of closed photobioreactor was soon faced with serious limitations because at a volume of 50–100 L it is no longer possible to effectively introduce the light energy required for successful biomass development. Several technical approaches to underwater lighting, for instance with submersed lamps or light diffusing optical fibers on the one hand, or with pillar-shaped photobioreactors on the other hand, have been tried, but have not been successful in application [Gerbsch et al., 2000; Semenenko et al., 1992]. However, this principle seems to be of future relevance only for the aquaculture of certain selected species.

Closed photobioreactors (**Figure 1-3**) are currently tested for microalgal mass cultures in the following configurations: tubular systems (glass, plastic, and bag), column system, flattened, plate-type systems, and ultrathin immobilized configurations. Vertical arrangements of horizontal running tubes or plates seem to be preferred for reasons of light distribution and appropriate flow.

Since about the 1990s, parameters such as species efficient light incidence into the photobioreactor lumen, light path, layer thickness, turbulence and O_2 release from the total

system volume have gained in importance (**Table 1-4**). Closed or almost closed systems based on very different design concepts have already been implemented and tested up to pilot scale. The latest developments seem to be directed toward photobioreactors of a tubular configuration or of the compact-plate type as well as combinations of these main design principles in order to distribute the light over an enlarged surface area [Tredici and Materassi. 1992; Gabel and Tsoglin. 2000].

Assuming that light for photosynthesis should be continuously available to the receptor in the microalgal cell, a lamination or other enlargement of the reactor surface directed toward the light source seems to be the prime aim. For microalgae this idea may include an appropriate lowering of net light energy supply for the suspension because of the significantly lower level of light saturation needed for these organisms. The basic principle of the laminar concept for thin layer plate or thin diameter tube photobioreactors mimics the leaves of higher plants. For instance a 100-year-old, 10 m high lime tree shading an area of 100 m^2 has a leaf surface area of more than 2,500 m^2. Expressed as a surface to volume ratio this amounts to a value of 2.5 m^2/m^3.

On the basis of these considerations, the trend toward developing closed photobioreactors as already described is paralleled by conceptions of the use of relatively thin light-exposed reactor lumina. The tube diameter in tubular photobioreactors is reduced significantly and laminar, plate-type configurations are strongly favored. The tubular or pipe design principle is the most important basis of completely or partially closed cultivators in plastic ducts and especially in glass or plastic tubes. The development of closed photobioreactors, which had intensified by the end of the 1980s, seems to be of significant future importance. Compared with laminar, plate-type systems, the tubular systems seem to have identical configuration potentials, especially in cases of vertical packing of horizontally oriented tubes [Broneske et al., 2000; Molina Grima et al., 2000] (**Table 1-5**).

A large number of closed photobioreactor systems have been developed and these avoid some of the problems connected with open system use, most notably better environmental control and fewer problems associated with contamination and grazing. The least complex and probably most widely used is the hanging sleeve. These are commonly used in aquaculture for the production of food for shrimp and mollusc larvae [McLellan et al., 1991] and also for polysaccharide production from *Porphyridium* [Becker, 1994]. Other types of bioreactors include tubular type reactors [Gudin and Chaumont, 1983; Torzillo et al., 1986],

laminar types [Tredici et al., 1991] and fermenter type reactors [Pohl et al., 1986] (**Figure 1-1**). Fermenter type reactors offer the greatest degree of control and may be fitted with light diffusing optical-fiber systems to avoid the necessity of external illumination and the problems associated with light limitation [Takano et al., 1992].

1.3.3. Comparison of open and closed culture systems for microalgae

Although significant progress has been made in finding a suitable microalga, there are still several major problems to over come in order to make the biological CO_2 reduction applicable is required for the system to do a meaningful CO_2 sequestration. For example, a raceway pond, the most widely used photobioreactor for commercial production of microalgae, requires 1.5 km^2 to fix the CO_2 emitted from a 150 MW thermal power plant [Karube et al., 1992]. Thus, it is important to maximize both volumetric productivity and photosynthetic efficiency to reduce the capacity of the system. However, it is not an easy task because the two objectives are, in part, contradictory. In most cases, the high cell concentration may decrease the photosynthetic efficiency because of the shadowing effects by the cells themselves. Many photobioreactors with various configurations, which may introduce light energy efficiently in to the dispersion of microalgae, have been developed.

As enclosed photobioreactors have many advantages over raceway ponds, such as higher productivity and possible application for various microalgae species, the application of the closed photobioreactor has been a focus of the R&D activity to develop microalgae greenhouse gas mitigation technologies. Thus, extensive work for the development of a highly efficient enclosed photobioreactor having a high aerial productivity and a low cost for construction has been done over the last few decades [Bayless et al., 2003; Pulz, 2001; Lee, 2001]. The aerial productivities of various photobioreactors have been compared in **Table 1-6**.

Several technical approaches to improve the light utilization efficiency of the reactor with higher cell densities have been tried by using Fresenel lenses and optical fibers [Takano et al., 1992; Michiki, 1995; Nishikawa et al., 1992]. Takano et al. reported that they could obtain CO_2 4.44 g/L/day with a cell concentration of 6.8 g/L [Takano et al., 1992]. The CO_2 removal rate is two or three times higher than that obtained at a small tubular reactor. The optical fiber reactor has not been in application because of the high capital cost. However,

this principle seems to be applicable only for the aquaculture of certain species in the future. Among various photobioreactors, the enclosed photobioreactor has the highest productivity based on capital cost. While many experimental photobioreactors have been designed, constructed and deemed successful, very few have been actually successful on the commercial scale [Olaizola, 2003; Hase et al., 2000].

Scaling up of the research photobioreactor to a commercial scale is not trivial. Problems related to the scale up of the photobioreactor were reviewed by Tredici [1999]. Currently only three commercial enclosed photobioreactor system, which consists of compact and verticalltarranged, horizontally running glass tubes of a total length of 500,000 m and a total reactor volume of 700 m^3. The system takes 10,000 m^2 for installation and 260~300 tons of CO_2 which results in the annual production of 130~150 tons dry biomass. Because the length of photobioreactor is too long, shear stress should be high. Therefore, the reactor may not be used for the cultivation of shear sensitive algal cells. The system is used for culturing *Chlorella*. Mera Pharmaceuricals, Inc. (formerlt Aquasearch Inc.) developed another horizontal enclosed photobioreactor suitable for the culturing of shear sensitive cells. The volume of the unit system is 25,000 L. The reactor is now used for the culturing of *Haematococcus pluvialis*. Dome-shaped photobioreactor has been developed and used for culturing *H. pluvialis* [Melis et al., 1999]. However, none of them are currently used for CO_2 mitigation.

Another important factor responsible for low productivity is the light saturation effect. Microalgae cultures can utilize only a fraction of the sun light to which they are exposed, typically one third or less. The reason for this is that the algal photosynthetic pigments capture more protons under full sunlight conditions than can be processed by photosynthesis. Recent research demonstrated that algal cultures and mutants with reduced antenna sizes can exhibit increased photosynthetic rates under high light intensities [Nakajima and Ueda, 2000].

1.4. Development of high efficient photobioreactor and utilization of microalgal cells produced.

CO_2 reduction by microalgae has emerged as a promising option for CO_2 mitigation. Intensive research work has been carried out to develop a feasible system for removing CO_2

from industrial exhaust gases. However, there is still several challenging point to overcome in order to make the process more practical. In this thesis, recent research activities on three key technologies of biological CO_2 reduction, an identification of a suitable algal strain, development of high efficient photobioreactor and utilization of algal cells produced, are described. Finally the barriers, progress, and prospects of commercially developing a biological CO_2 reduction process are summarized.

For the mass culture of microalgae, open pond systems have mainly been the dominating systems up until now. However, closed systems of light-distributing tube or plate design, known as photobioreactors, are now increasingly finding new applications both for high value products in pharmacy and cosmetics as well as for aqua- and agricultural uses.

Advancements in basic science with impact on the knowledge of the physiology, biochemistry, and molecular genetics of carotenoid-producing microalgae will also have a profound impact on the development of this and other microalgal-based processes and technologies.

II. Materials and Methods

2.1. Microalgal strain

The microalgae of *Chlorella* sp., *Nannochloropsis oculata*, *Skeletonema costatum*, *Isochrysis aff. galbana* and *Tetraselmis chui* were 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. This result of sequence alignment was performed by NCBI nucleotide blast [Wu et al., 2001]. *Chlorella* sp. used in this study is identified as several *Chlorella* sp. strain, such as KAS001, KAS005, KAS007, KAS012, MBIC10088, MDL5-18 and SAG 211-18.

2.2. Culture medium and chemicals

Microalgae, *Chlorella* sp., *Nannochloropsis oculata*, *Skeletonema costatum*, *Isochrysis galbana* and *Tetraselmis chui* were cultured in artificial sea water enriched with f/2 medium and an illumination of 300 μmol/m^2/s by white fluorescent light at 26 ± 1°C. Artificial sea water has following composition (per liter): including 29.23 g NaCl (Showa, Tokyo, Japan), 1.105 g KCl (Showa, Tokyo, Japan), 11.09 g $MgSO_4 \cdot 7H_2O$ (Amresco, Solon OH, USA), 1.21 g Tris-base (Merck, Darmstadt, Germany), 1.83 g $CaCl_2 \cdot 2H_2O$ (Amresco, Solon OH, USA), 0.25 g $NaHCO_3$ (Amresco, Solon OH, USA) (**Table 2-1**). f/2 medium (**Table 2-2**) has following composition (per liter): 75 mg $NaNO_3$ (Showa, Tokyo, Japan), 5 mg $NaH_2PO_4 \cdot H_2O$ (Sigma, Saint Louis, MO, USA), 1 mL of trace metal solution (**Table 2-3**), and 1 mL of vitamin solution (**Table 2-4**) [Guillard, 1975]. Trace elemental solution (per liter) includes 4.36 g $Na_2 \cdot$EDTA (Amresco, Solon OH, USA), 3.16 g $FeCl_3 \cdot 6H_2O$ (Sigma, Saint Louis, MO, USA), 180 mg $MnCl_2 \cdot 4H_2O$ (Sigma, Saint Louis, MO, USA), 10 mg $CoCl_2 \cdot 6H_2O$ (Sigma, Saint Louis, MO, USA), 10 mg $CuSO_4 \cdot 5H_2O$ (Sigma, Saint Louis, MO, USA), 23 mg $ZnSO_4 \cdot 7H_2O$ (Showa, Tokyo, Japan), 6 mg Na_2MoO_4 (Sigma, Saint Louis, MO, USA). Vitamin solution (per liter) includes 100 mg vitamin B_1 (Sigma, Saint Louis, MO, USA), 0.5 mg

vitamin B_{12} (Sigma, Saint Louis, MO, USA) and 0.5 mg biotin (Sigma, Saint Louis, MO, USA).

The microalgae were selected for the studies of CO_2 challenge and the high biomass concentration which were cultured in modified f/2 medium (**Table 2-5**) in artificial sea water at 26 ± 1°C with an illumination of 300 μmol/m^2/s by white fluorescent light.

2.3. Freezing procedure

Cryoprotective solution: cryprotectant agent was employed 1.1M glycerol. Glycerol was diluted in medium with f/2 solution. NaCl concentration was restricted to 340 mM in cryoprotective solution to prevent cells from experiencing excessive osmotic pressure.

Freezing procedure: 10 ml cultures (circa 5 × 10^6 cells/mL) were centrifuged (1000 × *g*, 10 min) to obtain an approximately ten-fold higher cell density. The cultures was resuspensed with cryoprotective solution in 2 mL cryotube and acclimatized at room temperature in 20 min. The cryotube was keeping the cooling rate at -3°C min^{-1} from room temperature to -40°C. A faster cooling rate (-8°C/min) was then applied down to -90°C, after which tubes were transferred to liquid N_2 [Poncet and Veron, 2003].

2.4. Thawing procedure

After storage periods (circa 1 month), cryotubes were extracted from the liquid N_2 and placed directly in a preheated water bath (30°C) until complete melting. Cells were gradually diluted ten-fold in f/2 solution. After 20 min equilibration at room temperature, cells were carefully washed in f/2 solution. The initial cell densities were adjusted to 5 × 10^6 cells/mL. Cultures were maintained for seven days in the growth conditions by white fluorescent light on a 14:10 h light/day cycle without aeration at 26°C. And then, cultures were maintained for culture condition above. [Poncet and Veron, 2003]

2.5. Microalgal culture system with photobioreactor

The microalgae was incubated in a cylindrical glass photobioreactor (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 2-1**. Cultures were placed on a bench at 26 ± 1°C under continuous, cool white, fluorescent light. Light intensity was supplied approximately 300 μmol/m^2/s at the surface of the photobioreactor. Gas provided as different concentrations of CO_2 mixed with ambient air were prepared with a volumetric percentage of CO_2 and filtered (0.22 μm) to give various CO_2 concentrations of 2, 5, 10, and 15%. The microalgal cultures were aerated continuously with gas provided via bubbling from the bottom of photobioreactor with an aeration rate of 200 mL/min (i.e., 0.25 vvm, volume gas per volume broth per min).

2.6. Preparation of the inoculum

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

2.7. Experimental design of batch cultivation

The photobioreactor was filled with 750 mL modified f/2 medium. The medium was aerated for 24 hr and then inoculated with 50 mL of precultured *Chlorella* sp. and *Nannochloropsis oculata* containing either 8×10^5 cells/mL (low density) or 8×10^6 cells/mL (high density). The cells from a 50 mL (at the density of 1×10^7 cells/mL) of precultured microalgae 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. Different CO_2 concentration was produced by mixing air and pure CO_2

at 0.25 vvm and adjusted by gas flow meter (Dwyer Instruments, Inc., Michigan, IN, USA) to give a flow rate of 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 hr, each culture was sampled to determine optical density, microalgal dry weight, and culture pH.

2.8. Experimental design of semi-continuous cultivation

The semi-continuous cultivation system was setup in a single photobioreactor and a system with six-parallel photobioreactor (**Figure 2-2**). 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 (the value of $A_{682} > 5$), half of volume of the culture broth was replaced with fresh modified f/2 medium every 24 hr and performed for 3 d. After that, In each photobioreactor, the culture was also replaced half of broth with fresh medium at the forth day and aerated with 2, 5, 10, and 15% CO_2 at 0.25 vvm. After 4 d culture, the sampling time was at 0, 6, 12 and 24 h everyday and the culture was replaced half of broth with fresh medium daily. 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.

2.9. 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) 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 if the optical density was greater than 1.0. Cell suspensions should be dilute enough so that the cells do not overlap each other on the

grid, and should be uniformly distributed. Systematically count the cells in selected squares so that the total count is approximate 300 cells.

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 if the optical density was greater than 1.0. Microalgal dry weight (g/L) 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°C for 16 hr [Takagi et al., 2006].

2.10. Analyses

2.10.1. Measurement of growth rate

A regression equation of the cell density and dry weight per liter of culture was obtained by a spectrophotometric method [Guillard, 1973; Chiu et al., 2008]. Biomass was calculated from microalgal biomass produced per liter (g/L). Specific growth rate (μ) was calculated as follows [Ono and Cuello, 2007]:

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

W_f: final biomass concentration

W_0: initial biomass concentration

$\triangle t$: cultivation time (days)

2.10.2. pH measurements

Sample pH was determined directly with an ISFET pH meter KS723 (Shindengen Electric Mfg.Co.Ltd, Tokyo, Japan). The pH meter was calibrated daily using pH 4 and 7 solutions.

2.10.3. Light measurements

Light intensity was measured from the light-attached surface of the photobioreactor using a Basic Quantum Meter (Spectrum Technologies, Inc., Plainfield, IL, USA).

2.10.4. 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).

2.11. 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.

III. Results and Discussion

3.1. Evaluation of cell density

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 were established by linear regression firstly. Optical density precisely predicted cell density *Chlorella* sp. ($R^2 = 0.997$; $p < 0.001$) (**Figure 3-1**), *Nannochloropsis oculata* ($R^2 = 0.995$; $p < 0.001$) (**Figure 3-2**), *Skeletonema costatum* ($R^2 = 0.998$; $p < 0.001$) (**Figure 3-3**), *Isochrysis aff. Galbana* ($R^2 = 0.992$; $p < 0.001$) (**Figure 3-4**) and *Tetraselmis chui* ($R^2 = 0.993$; $p < 0.001$) (**Figure 3-5**), respectively. Therefore, the values of optical density were used to calculate the related cell density of *Chlorella* sp., *Nannochloropsis oculata*, *Skeletonema costatum*, *Isochrysis aff. galbana* and *Tetraselmis chui* in each experiment according the equations established in this study.

3.2. The microalgae was screened for its potential ability of growth and biomass production

We attempted to study the growth capacities of microalgae via the controlling cultural environment and medial nutrition. Five microalgae, *Chlorella* sp., *Nannochloropsis oculata*, *Skeletonema costatum*, *Isochrysis galbana* and *Tetraselmis chui*, were used in this study. **Figure 3-6** was screened growth potential and biomass concentration of the microalgae. The optimal growth potential of *Chlorella* sp., *Nannochloropsis oculata*, *Skeletonema costatum*, *Isochrysis galbana* and *Tetraselmis chui* cultured in the f/2 medium at 26 ± 1°C and 300 $\mu mol/m^2/s$ for 24hr lighting with 0.25 vvm air aeration (designed as normal cultural medium) with air aeration were 1.55, 1.51, 0.5, 0.72 and 0.99 d^{-1}, respectively. The maximum cell density of *Chlorella* sp., *Nannochloropsis oculata*, *Skeletonema costatum*, *Isochrysis galbana* and *Tetraselmis chui* cultured in the f/2 medium (designed as normal cultural medium) with air aeration were 4.7×10^7, 5.5×10^7, 1.9×10^7, 2.1×10^7,and 1.9×10^7 cells/mL, respectively. *Chlorella* sp. and *Nannochloropsis oculata* were selected from the study because of the higher growth potential and cell density. And then *Chlorella* sp. and *Nannochloropsis oculata* were

selected for the studies of CO_2 challenge and the high biomass concentration.

3.3. Evaluation of biomass concentration

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 dry weight were established by linear regression firstly. Optical density precisely predicted biomass *Chlorella* sp. ($R^2 = 0.992$; $p < 0.001$) (**Figure 3-7**) and *Nannochloropsis oculata* ($R^2 = 0.999$; $p < 0.001$) (**Figure 3-8**). Therefore, the values of optical density were used to calculate the related biomass of *Chlorella* sp., *Nannochloropsis oculata*, in each experiment according the equations established in this study.

3.4. Chlorella sp. *culture at different cell density aerated with different CO_2 concentration*

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°C and 300 μmol/m^2/s for 24hr lighting 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 concentration in air, 2% and 5% CO_2 aeration with low-density biomass inoculum 0.01 g/L (i.e., 8×10^5 cells/mL) were 0.537 ± 0.016, 1.211 ± 0.031, and 0.062 ± 0.027 g/L, respectively. Areation of air (CO_2 concentration is approximate 0.03%), 2, 5, 10 and 15% CO_2, the microalgae culture medium pH was 9.8, 7.8, 6.5, 6.1, 5.8, respectively. At the aeration of 2% CO_2, *Chlorella* sp. increased most rapidly at the specific growth rate of 0.492 μ, and the specific growth rate markedly fell to be 0.127 μ 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 and low pH;

therefore; the specific growth rates were not available (**Figure 3-9A** and **Table 3-1**). At 2% CO_2, growth of *Chlorella* sp. became stable after 6-8 days of incubation. Optical density at A_{682} was greater than 5 and biomass was greater than 1.0 g/L.

In the cultures inoculated with *Chlorella* sp. at high-density 0.1 g/L (i.e., 8×10^6 cells/mL), 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 pH, maximum biomass concentration and specific rate at 5% CO_2 aeration in high-density inoculum was 7.6, 0.899 ± 0.003 g/L and 0.343 μ, respectively. The values were significantly increased as compared with those in low-density inoculum. However, the growth of *Chlorella* sp. was inhibited after 3 days of incubation under the conditions of 10% and 15% CO_2 aeration (**Figure 3-9B** and **Table 3-1**). At 2% and 5% CO_2, a short lag period was observed in cultures with high-density inoculum but not in cultures with low-density inoculum. *Chlorella* sp. grew slowly at 10% and 15% CO_2. Optical density at A_{682} was less than 1.5. Moreover, at 10% and 15% CO_2, growth was inhibited after 3 days of incubation. After 6 days of incubation at 2% CO_2, growth reached a plateau and biomass was over 1.3 g/L. 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 reports that the waste gas or CO_2 tolerance of microalgae was dependent on cell density [Yoshihara et al., 1996; Yun et al., 1997; Lee et al., 2002].

3.5. Nannochloropsis oculata culture at different cell density aerated with different CO_2 concentration

Effect of CO_2 concentration in airstream on the growth of *Nannochloropsis oculata* was investigated in a batch culture incubated at 26 ± 1°C and 300 μmol/m^2/s for 24 hr lighting and with 0.25 vvm aeration. The initial biomass inoculum was 0.01 g/L (about 7×10^5 cells/mL) 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 3-10** 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 *Nannochloropsis oculata* were 0.268 ± 0.022 and 1.277 ± 0.043 g/L, respectively. The final pH was 9.8, 7.7, 6.4, 6.1 and 5.8 at air, 2, 5, 10, 15% CO_2 aeration, 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 μ and 0.571 μ, respectively. The culture aerated with 2% CO_2 showed an optimal growth potential. When the *Nannochloropsis oculata* 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].

3.6. Effect of CO_2 on Chlorella sp. in semi-continuous cultivation

3.6.1. Effect of CO_2 on cell growth in semi-continuous culture

The semi-continuous culture was carried out in two stages. A batch culture had an initial cell density of 8×10^6 cells/mL (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), 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 semi-continuous culture was constantly similar at 2, 5, 10, and 15% CO_2 (**Figure 3-11**). The average specific growth rate and biomass, respectively, were 0.58 to 0.66 μ and 0.76 to 0.87 g/L after 8 days of incubation at 2% to 15% CO_2 aeration. These semi-continuous 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 semi-continuous 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/d. The other mutant, *Chlorella* strain KR-1, showed a potential biomass of 1.1 g/L/d 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 [Yun et al., 1997; Lee et al., 2002]. In our semi-continuous 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.

3.6.2. Effect of CO_2 on biomass production in semi-continuous culture

Biomass productivity in the semi-continuous *Chlorella* sp. cultures were determined before the culture broth was changed each day. **Table 3-2** summarizes the results, biomass productivity, collected from the single photobioreactor and the six-parallel photobioreactor cultures under different CO_2 aeration. As a daily 50% culture broth replaced in the 800 mL semi-continuous photobioreactor aerated with 2, 5, 10, and 15% CO_2, the total biomass productivity per day (400 mL of culture broth was recovered for measurement) of each photobioreactor was 0.421, 0.404, 0.366 and 0.361 g/L/d, respectively. In the single semi-continuous culture, biomass productivity decreased when the aerated CO_2 concentration was increased. In the semi-continuous culture, the optimum condition for biomass productivity was at 2% CO_2 aeration and 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 biomass productivity in the semi-continuous system even the cells were cultivated in the condition aerated with 15% CO_2.

3.7. CO_2 reduction by Chlorella sp. in semi-continuous culture

Semi-continuous *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 difference in CO_2 concentration between the influent load and effluent load were monitored in the semi-continuous *Chlorella* sp. cultures during an 8-day period on 300 μmol/m^2/s for 24hr lighting at 26 ± 1°C (**Figure 3-12**). The influent and effluent CO_2 concentrations in each culture were measured at 6, 12, and 18 hr after the cultured broth was replaced each day. 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/hr, respectively (**Figure 3-13**). Thus, the overall efficiency of CO_2 reduction in the cultures was 58, 27, 20 and 16%, respectively (**Figure 3-14**). Recently, de Morais and Costa [2007b] reported greater efficiency of CO_2 reduction 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 reduction [Cheng et al., 2006]. Keffer and Kleinheinz [2002] demonstrated that air dispersed in photobioreactors operated under approximately 2 sec 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 sec 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.. Initial culture medium pH was between 8.0-8.2. Stably average pH was 7.6, 7.4, 7.1 and 6.8 at 2, 5, 10 and 15% CO_2 aeration, respectively. Free CO_2 concentration 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 reduction in a closed culture system is dependent on the microalgal species, CO_2 concentration, and photobioreactor [Chen et al., 2006; de Morais and Costa, 2007]. Cheng et al. [2006] have demonstrated that maximum CO_2 removal efficiency (55.3%) at 0.15% CO_2 and the maximum CO_2 reduction rate (about 80 mg/L/hr) 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 reduction efficiency was only 7–17% for *Spirulina* sp. and 4–9% for *S. obliquus* [de Morais and Costa, 2007]. The species dependence of efficiency of CO_2 removal or reduction may be due to physiological conditions of microalgae, such as potential of cell growth and ability of CO_2 metabolism.

3.8. Performance of six-parallel photobioreactor system with Chlorella sp.

The efficiency of CO_2 removal from airstreams by *Chlorella* sp. was compared between the single photobioreactor and the six-parallel photobioreactor. Both photobioreactor systems were made of cylindrical glass photobioreactor with 30 cm in length and 7 cm in diameter. Each unit of photobioreactor contained 800 mL cultured microalgae. 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/hr at 2, 5, 10 and 15% CO_2 aeration, respectively (**Figure 3-13**). 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 3-14**).

Daily recovery of biomass in the six-parallel photobioreactor was determined. In each case, the amount of biomass recovered daily in the six-parallel photobioreactor was around six times greater than the amounts recovered in the single photobioreactor (**Table 3-3**). 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 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.

3.9. CO_2 utilization of Nannochloropsis oculata

3.9.1. Effect of CO_2 concentration on cell growth in semi-continuous cultures

For the study of biomass productivity in response to higher CO_2 aeration, the microalgal cells pre-adapted to CO_2 were applied. In the experiment, *Nannochloropsis oculata* cells were pre-adapted to 2% CO_2 before the microalga was inoculated into the semi-continuous cultures. Moreover, a high density (approximate 0.4 g/L) of inoculum was applied in the cultures. The semi-continuous 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 *Nannochloropsis oculata* aerated with 2, 5, 10, and 15% CO_2 in the semi-continuous system were similar. The average specific growth rate and maximum cell density (i.e., biomass concentration) were from 0.683 to 0.733 μ and from 0.745 to 0.928 g/L at different concentrations of CO_2 aerated cultures, respectively (**Figure 3-15**). High CO_2 aeration (5-15%) may be a harmful effect on the microalgal cells growth as shown in **Figure 3-10**. 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.

3.9.2. Optimal CO_2 concentration applied in semi-continuous cultures

In the semi-continuous system, *Nannochloropsis oculata* 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 semi-continuous system cultured with *Nannochloropsis oculata* 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 reduction, and CO_2 removal efficiency were recorded and showed in **Figure 3-16**. 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/hr, 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, 2007b; 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, 2007]. 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 reduction 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 0.211, 0.234, 0.350 and 0.393 g/hr, however, total biomass productivity was 0.480, 0.441, 0.398 and 0.372 g/L/d 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 productivity was 2% CO_2.

3.10. Biomass production of Nannochloropsis oculata in semi-continuous culture

In the semi-continuous culture system, the *Nannochloropsis oculata* cells were collected at the time before culture replaced each day for determination of biomass productivity. **Table 3-4** summarizes the biomass productivity of *Nannochloropsis oculata* cultures aerated with various CO_2 concentrations. As increasing CO_2 concentration of aeration from 2 to 15%, both biomass productivity were generally decreasing. Our results showed 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 biomass production was decreased with decrease of pH. This may be possibly because the higher pH having higher available bicarbonate for carboxylation of biomass production. This inference supports the result that biomass production in *Nannochloropsis oculata* may be affected by pH and biomass synthesis of the microalgal cultures was decreased with decrease of broth pH.

A six-parallel photobioreactor system was also performed in this study, and the CO_2 reduction efficiency in the system was similar to the single photobioreactor in different

concentrations of CO_2 aeration. Daily recovery of biomass productivity of *Nannochloropsis oculata* in the six-parallel photobioreactor was determined. The amount of biomass recovered daily in the six-parallel photobioreactor was around six times greater than the amounts recovered in the matched single photobioreactor (**Table 3-4**). CO_2 reduction efficiency and cell growth in both photobioreactor systems also were similar.

The comparison of productive efficiencies in the semi-continuous systems in which the culture broth were replaced at an interval of 24 hr (one-day replacement) or 72 hr (three-day replacement) was performed. In the systems, approximate 0.4 g/L of *Nannochloropsis oculata* 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 semi-continuous system after the cultures aerated with 2% CO_2. **Figure 3-17** shows the stable growth profiles of *Nannochloropsis oculata* 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. 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 biomass productivity of *Nannochloropsis oculata* cultures in the semi-continuous culture system with one-day and three-day replacement was 0.497 ± 0.032 and 0.296 ± 0.009, respectively. The total biomass in the three-day replacement cultures were only 24% 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. In conclusion, the total biomass in the semi-continuous culture operated by one-day replacement was more efficient compared with those in three-day replacement.

IV. Conclusions

4.1. The microalgae was screened for its potential ability of growth and biomass production

Chlorella sp. and *Nannochloropsis oculata* were selected from this studies. The results show that through air aeration of 0.25 vvm into the microalgal culture, *Chlorella* sp. and *Nannochloropsis oculata* have higher growth potential and maximal cell density than *Skeletonema costatum*, *Isochrysis aff. galbana* and *Tetraselmis chui*.

4.2. Reduction of CO_2 by Chlorella sp. and Nannochloropsis oculata in semi-continuous photobioreactor

Our results show that airstreams containing a high concentration of CO_2 (2 to 15%) may be introduced directly into a high-density culture of *Chlorella* sp. in a semi-continuous photobioreactor. Growth, biomass productivity, lipid productivity, and the quantity of CO_2 removed from the airstreams remained constant in the photobioreactor. The maximum efficiency of CO_2 reduction reached 58% in the culture aerated with 2% CO_2. The greatest reduction rate of CO_2 was 17.2 g/L/d occurred at 15% CO_2. These productivity and efficiency of CO_2 reduction did not decrease when a parallel (multiple units) photobioreactor was used.

4.3. Biomass productivity of Chlorella sp. *and Nannochloropsis oculata in semi-continuous photobioreactor*

The microalgae pre-adapting to 2% CO_2 cultured in a semi-continuous system with a high cell density of inoculums could grow well in the system aerated with higher CO_2 concentration (5-15% CO_2); however, increasing biomass production would not be followed as the cultures aerated with higher CO_2. Achieving the optimal condition for a long-term

biomass in the semi-continuous system, the microalga could be cultured with 2% CO_2 aeration in one-day replacement operation.

4.4. Photobioreactor design

The photobioreactor is an air-lift type photobioreactor with sparger for high-density microalgal culture. The photobioreactor was used for a long-term maintained high-density culture of microalgae in a semicontinuous culturing operation. The capacity of daily biomass production of the microalga was increment, obviously. The maximum biomass productivity could reach to approximately 0.4 g/L/d in half volume of broth replacement every days and increase the efficiency of CO_2 removal.

4.5. Six-parallel photobioreactor system

The efficiency of CO_2 removal was similar in the single photobioreactor and in a six-parallel photobioreactor. However, CO_2 reduction and production of biomass were six times greater in the six-parallel photobioreactor than in the single photobioreactor. Therefore, biological reduction of CO_2 and biomass production in the established system could be parallely increased using the photobioreactor consisting of parallel systems.

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Table 1-1. The chemical method of carbon dioxide fixation

Method	The absorbent
Activated MEDA	MEDA
Amine Guard FS	MEA
Benfield, Carsol, Catacarb and Flexsorb	Hot potassium Carbonate
Fluor	Propylene Carbonate
Purisol	N-methy-pyrrolidone
Rectisol	Cold Methanol
Selexol	Dimethyl ether of polyethylene glycol
Sulfinol	Alkanolamine and tetrahydrothiophene diocide

Table 1-2. Chemical composition of algae expressed on a dry matter basis (%) [Becker, 1994]

Strain	Protein	Lipid	Nucleic acid	Carbohydrates
Scenedesmus obliquus	50-56	12-14	3-6	10-17
Scenedesmus quadricauda	47	1.9	-	-
Scenedesmus dimorphus	8-18	16-40	-	21-52
Chlamydomonas rheinhardii	48	21	-	17
Chlorella vulgaris	51-58	14-22	4-5	12-17
Chlorella pyrenoidosa	57	2	-	26
Spirogyra sp.	6-20	11-21	-	33-64
Dunaliella bioculata	49	8	-	4
Dunaliella salina	57	6	-	32
Euglena gracilis	39-61	14-20	-	14-18
Prymnesium parvum	28-45	22-38	1-2	25-33
Tetraselmis maculata	52	3	-	15
Porphyridium cruentum	28-39	9-14	-	40-57
Spirulina platensis	46-63	4--9	2-5	8-14
Spirulina maxima	60-71	6-7	3-4.5	13-16
Synechoccus sp.	63	11	5	15
Anabaena cylindrica	43-56	4-7	-	25-30

Table 1-3. Product from microalgae

Potential use	Strain	Product
Antitumor agent	*Amphidinium* sp.	Amphidinolides Amphdinins
	Alexandrium hiranoi	Goniodomins
Pigment	*Haematococcus pluvialis*	Astaxanthin
	Chlorella sp.	Astaxanthin
Colorant	*Dunaliella*	*β*-Carotene
	Red algae	Phycobiliproteins
	Cyanobacteria	Phycobiliproteins
	Spirulina platensis	Phycocyanin
Food supplement	*Dunaliella*	*β*-Carotene
Essential fatty acid	*Isochrysis galbana*	DHA
	Spirulina sp.	*γ*-Linolenic acid
Health care	*Phaeodactylum tricornutum*	PUFA
Antioxidant	*Phaeodactylum tricornutum*	Fucoxanthin
Elastase inhibitor	*Oscillatoria agardhii*	Oscillapeptin

Table 1-4. 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 control	Easy	Difficult
Area/volume ratio	High	Low
Population density	High	Low
Process control	Easy	Difficult
Investment	High	Low
Operation costs	High	Low
Light utilization efficiency	Excellent	Poor

Table 1-5. Basic values of various cultivation plants (German cultivation sites at natural illumination)

	Unit	Raceway	Open pond	Tubular	Plate (Semi-closed system)
Illuminated surface	m^2	500	200	600	500
Total volume	m^3	75	5	7	6
Space required	m^2	550	250	110	100
Layer thickness	cm	10 – 30	0.5 – 1	4	3
Flow rate	cm/s	30 – 55	30 – 45	50 – 60	120
Biomass conc.(DW)	mg/L	300 – 500	3,000 – 6,500	5,000 – 8,000	5,000 – 8,000
Productivity (DW)	g/L/d	0.05 – 0.1	0.8 – 1.0	0.8 – 1.2	0.8 – 1.3

Table 1-6. Aerial 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
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
Flat plate	Vertical	1.5	Japan	*Synechocytis aquatilis*	31.0		Zhang et al., 2001
Flat plate	Vertical continuous	10		*Nannochloropsis* sp.	12.0		Zhang et al., 2001
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
Annular	Inclined	3.5		*Nannochloropsis* sp.	52.5		Zittelli et al., 2003

Table 2-1. Artificial sea water

Chemical	Content (g/L)
NaCl	29.23
$CaCl_2 \cdot 2H_2O$	1.83
KCl	1.11
$MgSO_4 \cdot 7H_2O$	11.09
Tris-base	1.21
$NaHCO_3$	0.25

Table 2-2. f/2 culture medium [Guillard, 1975]

Chemical	Stock solution (g/100 ml)	Nutrient solution (in 1000 ml filtered seawater)
$NaNO_3$	7.5	1 mL
$NaH_2PO_4 \cdot H_2O$	0.5	1 mL
$Na_2SiO_3 \cdot 9H_2O$ [a]	3.0	1 mL
Micronutrient working stock solution [b]		1 mL
Vitamin working stock solution [c]		1 mL

[a] Recommended for diatom culture.
[b] Refer to **Table 2-3**
[c] Refer to **Table 2-4**

Table 2-3. Preparation of micronutrient solution

Chemical	Primary stock solution (g/100 ml dist.H_2O)	Working stock solution (in 1000 ml dist. H_2O)
$CuSO_4 \cdot 5H_2O$	1.0	1 mL
$ZnSO_4 \cdot 7H_2O$	2.3	1 mL
$CoCl_2 \cdot 6H_2O$	1.0	1 mL
$MnCl_2 \cdot 4H_2O$	18.0	1 mL
$Na_2MoO_4 \cdot 2H_2O$	0.6	1 mL
Na_2EDTA		4.36 g
$FeCl_3 \cdot 6H_2O$		3.16 g

Table 2-4. Preparation of the vitamin solution

Chemical	Primary stock solution (mg/mL dist. H_2O)	Working stock solution (in 1000 mL dist. H_2O)
Vitamin B_{12}	0.5	1 mL
Biotin (hygroscopic, weigh approx. 10 mg and add 9.6 ml dist. H_2O/mg biotin)	0.5	1 mL
Thiamin HCl	100	1 mL

Sterilize by filtration or - slightly acidified with HCl - by autoclaving. vitamin working stock was added to nutrient solution after autoclaving.

Table 2-5. The modified f/2 culture medium

Chemical	Stock solution (g/100 mL)	Nutrient solution (in 800 mL filtered seawater)
$NaNO_3$	7.5	3 mL
$NaH_2PO_4 \cdot H_2O$	0.5	3 mL
$Na_2SiO_3 \cdot 9H_2O$ [a]	3.0	3 mL
Micronutrient working stock solution [b]		3 mL
Vitamin working stock solution [c]		1 mL

[a] Recommended for diatom culture.
[b] Refer to **Table 2-3**
[c] Refer to **Table 2-4**

Table 3-1. 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	Max. biomass concentration (cell dry weight, g/L)[b, c]	Specific growth rate (μ)[d]
Low-density inoculum (8×10^5 cells/mL)		
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)		
Air[a]	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 3-2. The biomass production and the specific growth rate of the low- and high-density inoculums of *Nannochloropsis oculata* growth depending on different concentrations of CO_2 aeration

CO_2 aeration	Max. biomass concentration (cell dry weight, g/L)[b, c]	Specific growth rate (μ)[d]
Low-density inoculum (8×10^5 cells/mL)		
Air[a]	0.263 ± 0.001	0.070
2%	1.280 ± 0.020	0.101
5%	0.073 ± 0.047	0.011
10%	0.011 ± 0.021	-
15%	0.010 ± 0.007	-
High-density inoculum (8×10^6 cells/mL)		
Air[a]	0.559 ± 0.004	0.117
2%	1.473 ± 0.012	0.533
5%	1.004 ± 0.037	0.161
10%	0.153 ± 0.023	-
15%	0.118 ± 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 3-3. Daily recovery of biomass productivity of the *Chlorella* sp. as waste broth in the semi-continuous photobioreactor under different concentrations of CO_2 aeration compared with single and six-parallel photobioreactor

CO_2 aeration	Biomass productivity of *Chlorella* sp. (cell dry weight, g/L/d)	
	In single photobioreactor[a]	In six-parallel photobioreactors[b]
2%	0.421 ± 0.059	2.534 ± 0.369
5%	0.404 ± 0.049	2.494 ± 0.235
10%	0.366 ± 0.044	2.259 ± 0.200
15%	0.361 ± 0.057	2.226 ± 0.313

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

Table 3-4. Daily recovery of biomass productivity of the *Nannochloropsis oculata* as waste broth in the semi-continuous photobioreactor under different concentrations of CO_2 aeration compared with single and six-parallel photobioreactor

CO_2 aeration	Biomass productivity of *Nannochloropsis oculata* (cell dry weight, g/L/d)	
	In single photobioreactor[a]	In six-parallel photobioreactors[b]
2%	0.480 ± 0.029	2.901 ± 0.097
5%	0.441 ± 0.044	2.784 ± 0.182
10%	0.398 ± 0.069	2.522 ± 0.256
15%	0.372 ± 0.022	2.438 ± 0.114

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

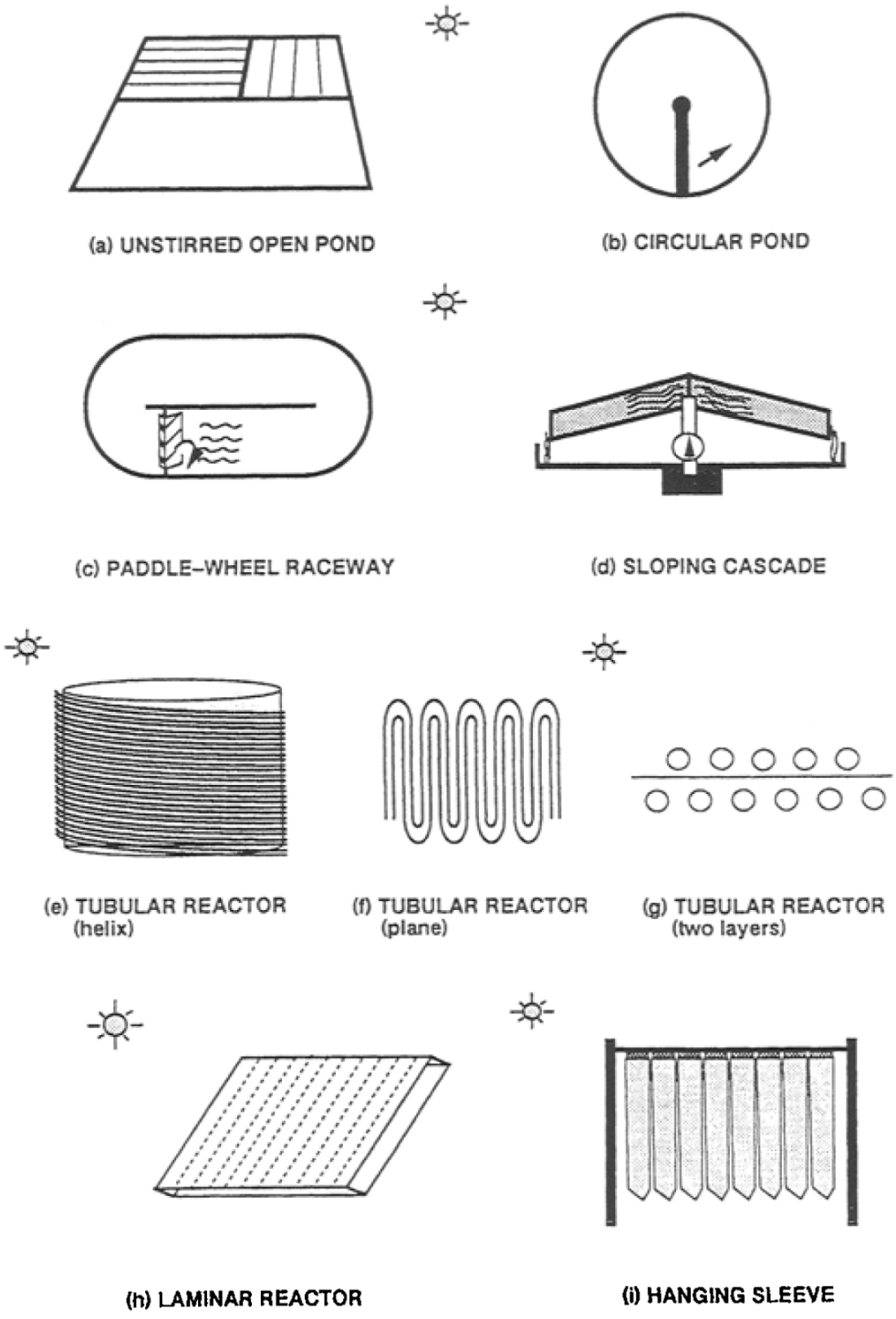


Figure 1-1. Typical examples of different algal mass culture systems used in the aquaculture and biotechnology industries. [Day et al., 1999]

Figure 1-2. Typical open pond production site using a raceway arrangement. (In Israel)

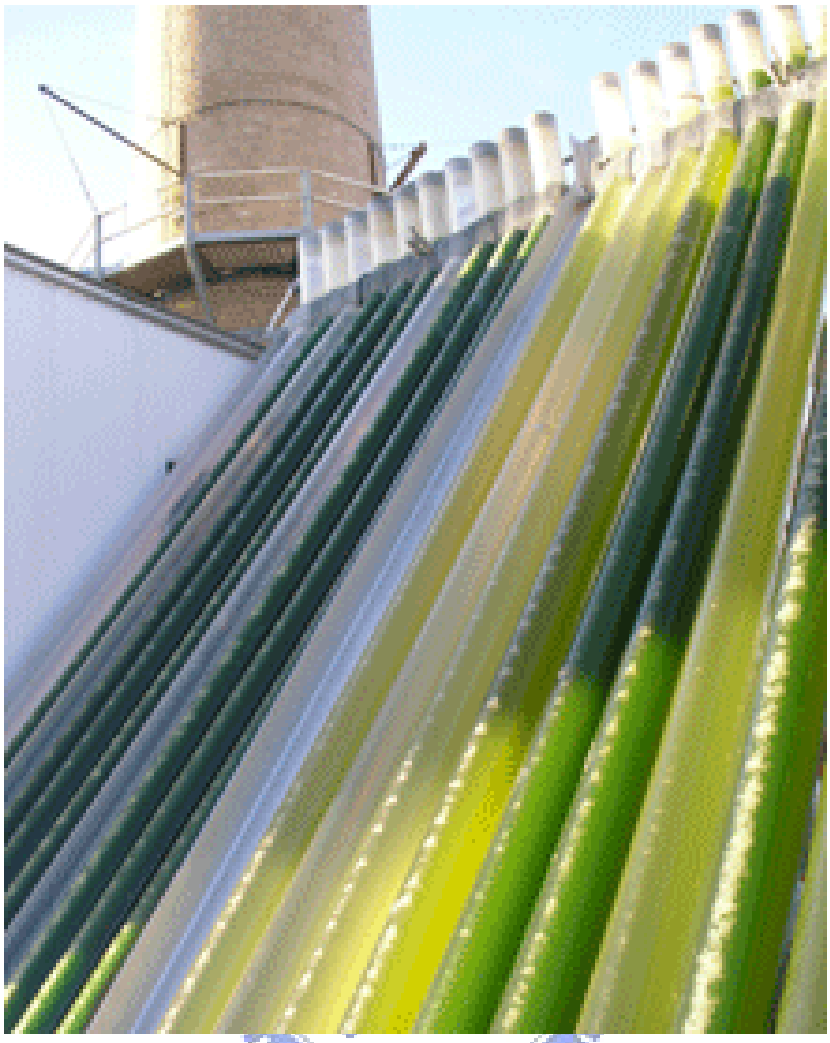


Figure 1-3. Closed column type photobioreactor fed with recycled CO_2 from smokestack, fermentation, and geothermal gases production plant in MA, USA.

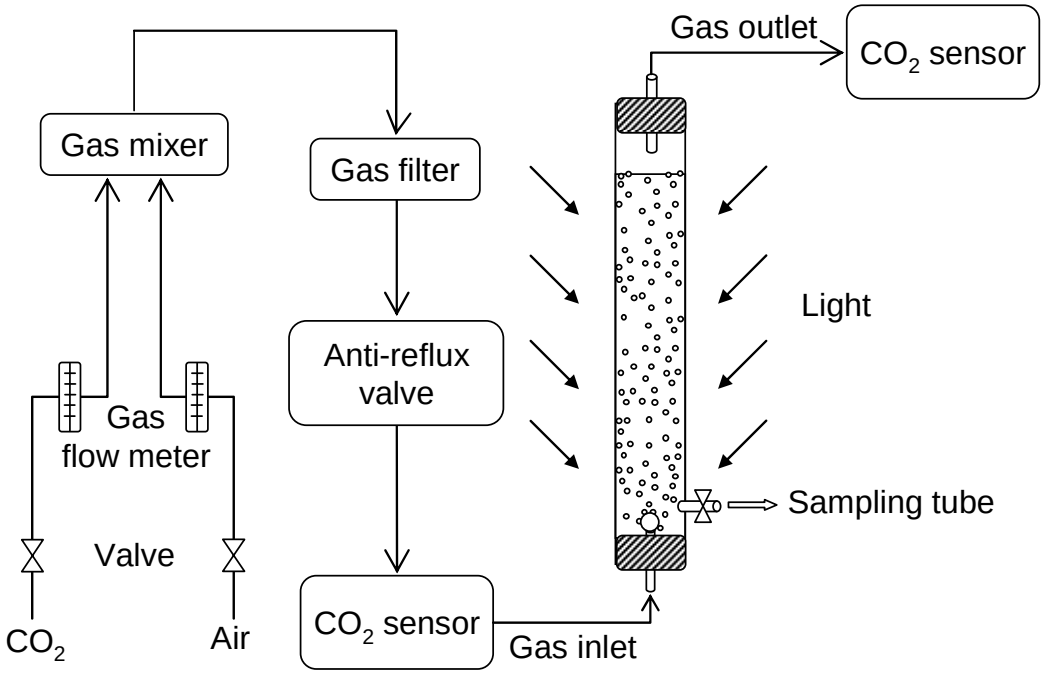


Figure 2-1. Schematic diagram of the photobioreactor for the experiments on CO_2 reduction for batch and semi-continuous microalgal cultures. The airstreams with different CO_2 concentrations was adjusted by individual gas flow meter and determined via CO_2 sensor. External illumination of light intensity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

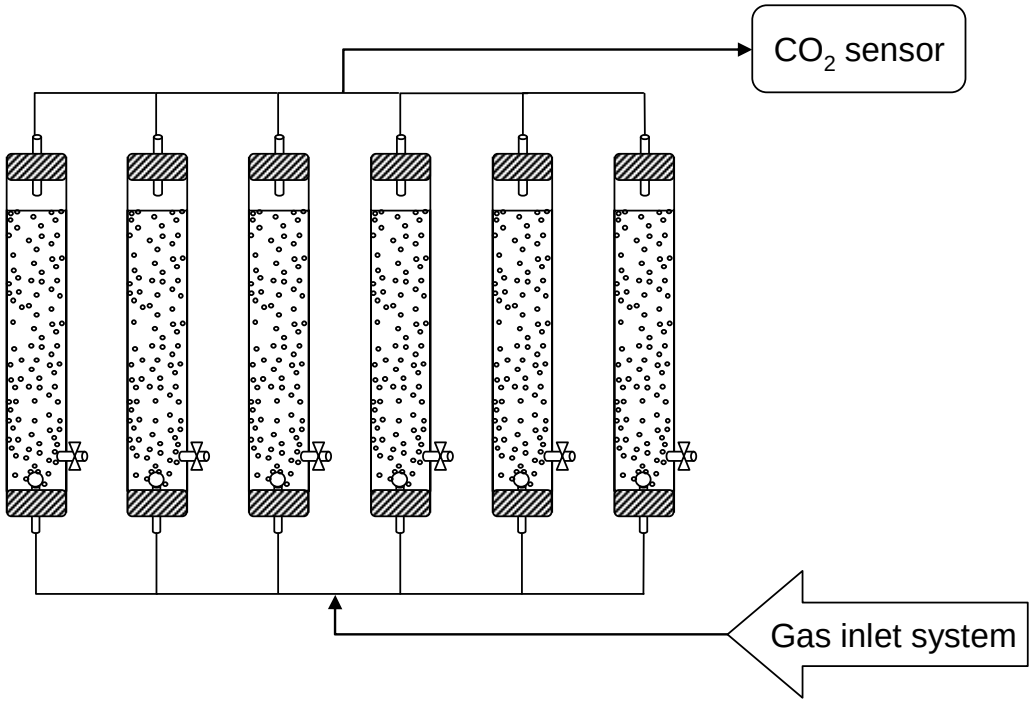


Fig. 2-2. Schematic diagram of the six-parallel photobioreactor for the experiments on biomass production and CO_2 reduction with semi-continuous microalgal culture. The airstreams with different CO_2 concentrations was adjusted by individual gas flow meter and determined via CO_2 sensor. External illumination of light intensity was provided with 300 μmol/m^2/s by a continuous, cool white, and fluorescent light.

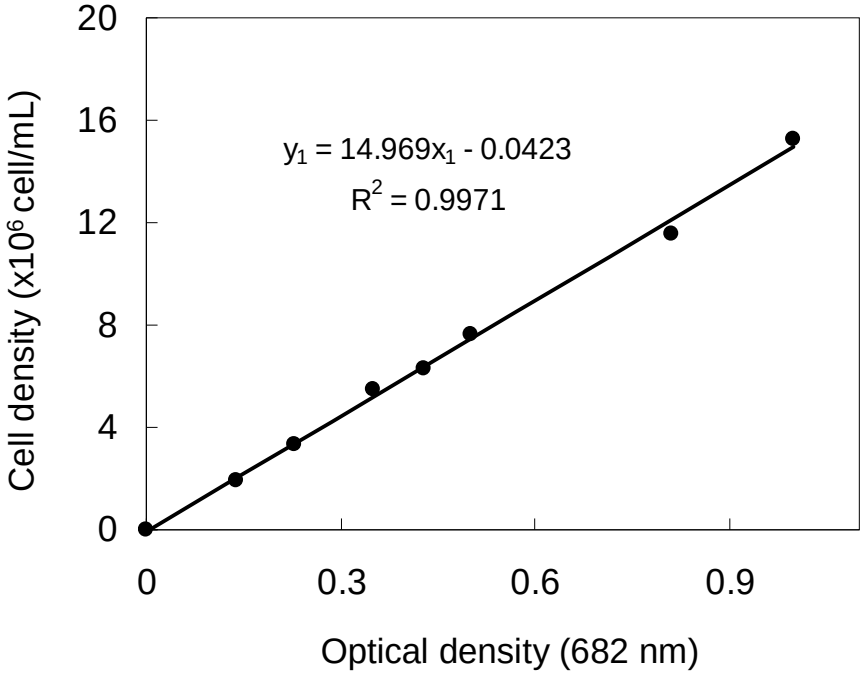


Figure 3-1. Calibration curves and equations of optical density of *Chlorella* sp. at A_{682} to the cell density (cells directed counted by microscope). The calibration curve, correlative equation and R^2 value of each correlation were indicated.

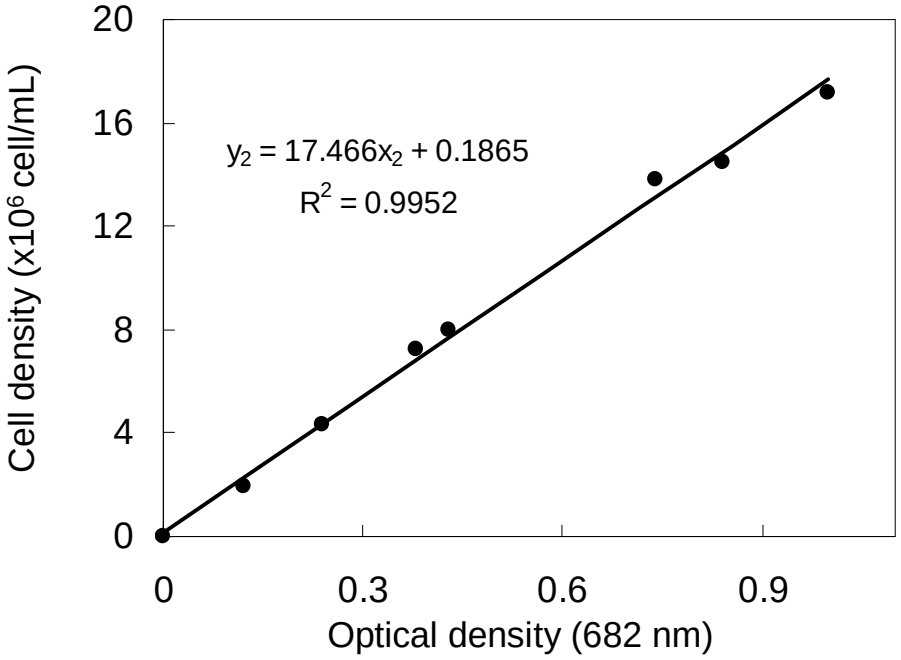


Figure 3-2. Calibration curves and equations of optical density of *Nannochloropsis oculata* at A_{682} to the cell density (cells directed counted by microscope). The calibration curve, correlative equation and R^2 value of each correlation were indicated.

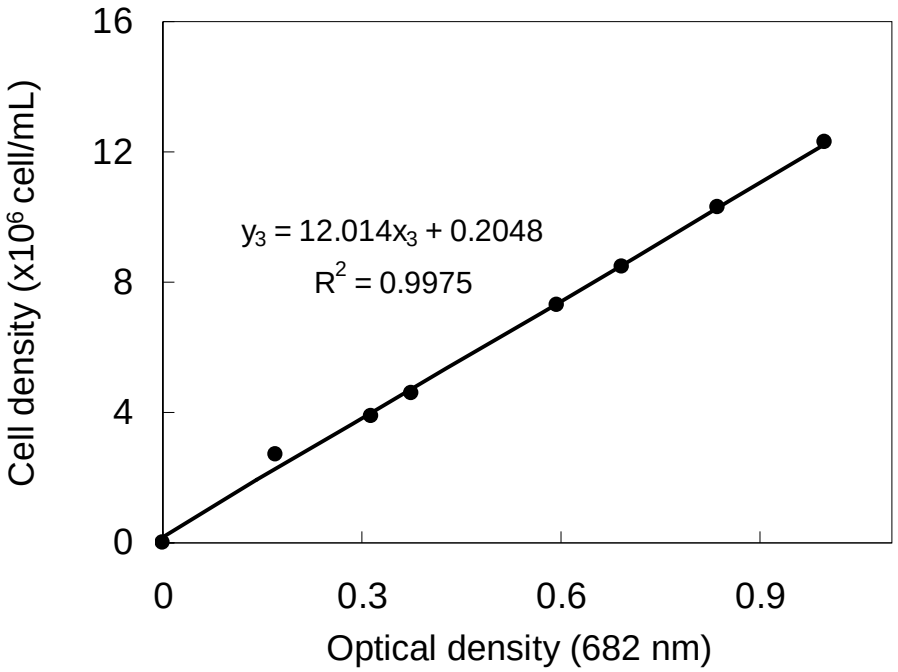


Figure 3-3. Calibration curves and equations of optical density of *Skeletonema costatum* at A_{682} to the cell density (cells directed counted by microscope). The calibration curve, correlative equation and R^2 value of each correlation were indicated.

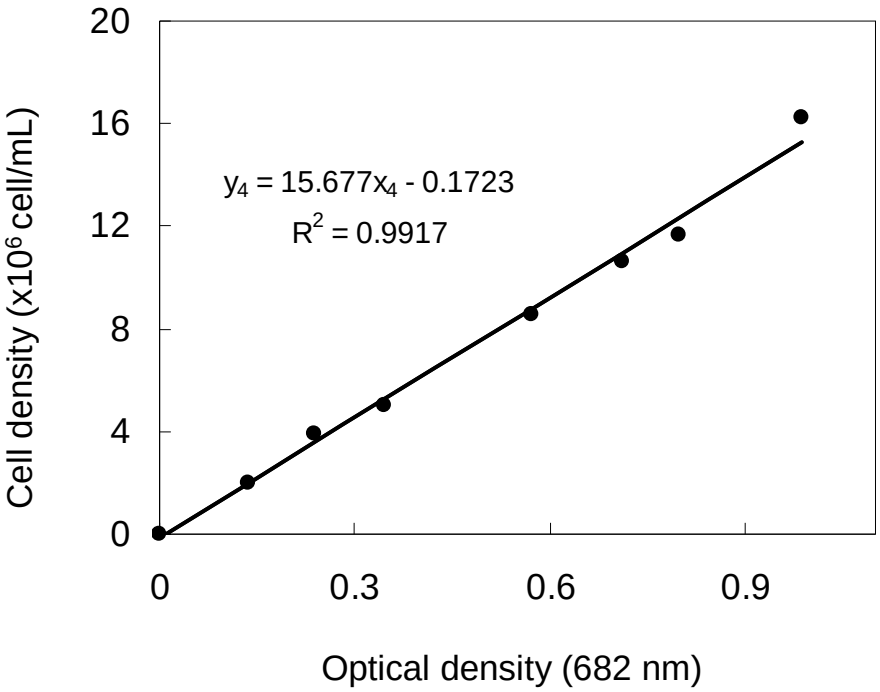


Figure 3-4. Calibration curves and equations of optical density of *Isochrysis aff. galbana* at A_{682} to the cell density (cells directed counted by microscope). The calibration curve, correlative equation and R^2 value of each correlation were indicated.

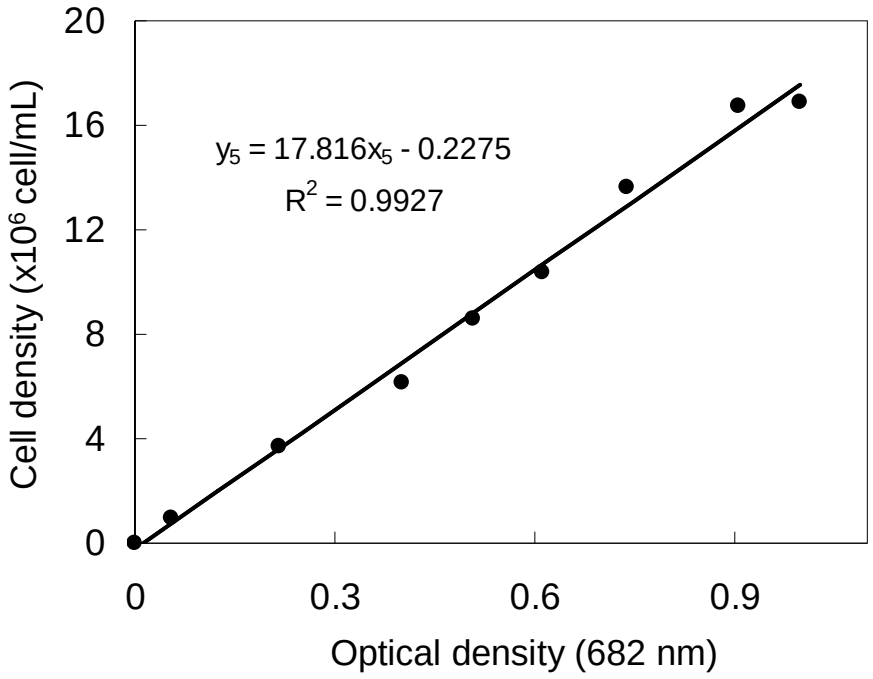


Figure 3-5. Calibration curves and equations of optical density of *Tetraselmis chui* at A_{682} to the cell density (cells directed counted by microscope). The calibration curve, correlative equation and R^2 value of each correlation were indicated.

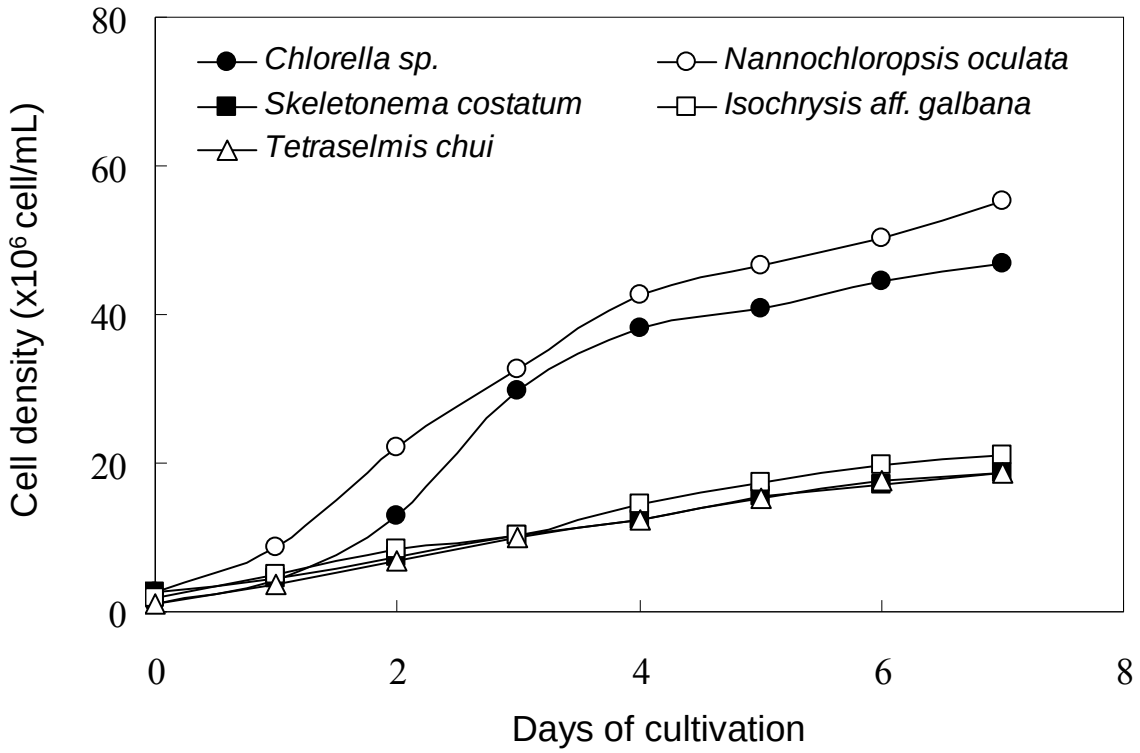


Figure 3-6. The growth curve of different microalgae, *Chlorella* sp. (●), *Nannochloropsis oculata.* (○),*Skeletonema costatum* (■), *Isochrysis aff. galbana* (□), *Tetraselmis chui* (△). The microalgal cell were screened with batch culture in modified f/2 medium at 26 ± 1°C with 0.25 vvm air areation. Light intensity was approximately 300 μmol/m^2/s for 24hr lighting.

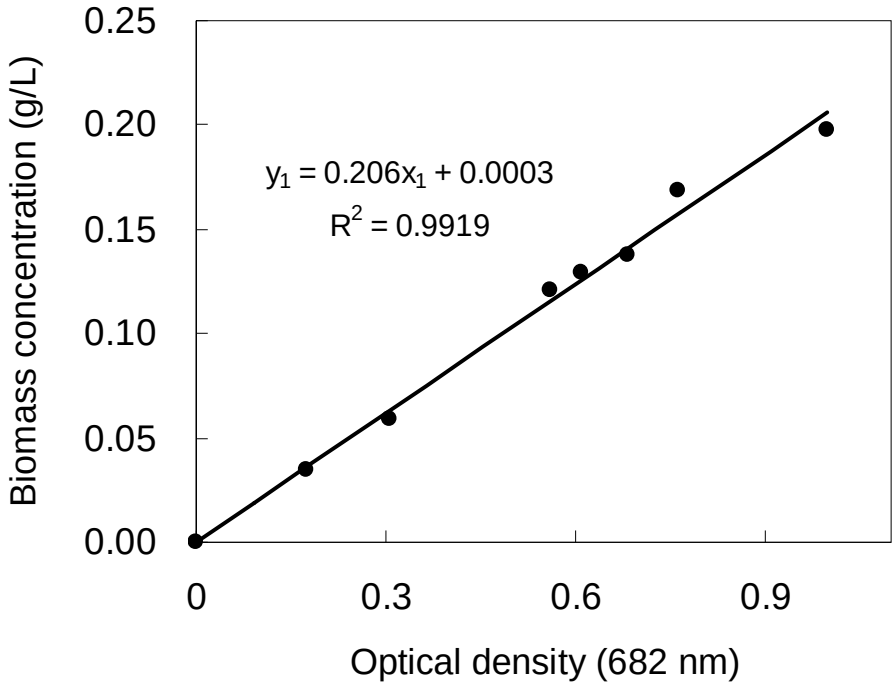


Figure3-7 Calibration curves and equations of optical density of *Chlorella* sp. at A_{682} to the biomass concentration (g/L). The calibration curve, correlative equation and R^2 value of each correlation were indicated.

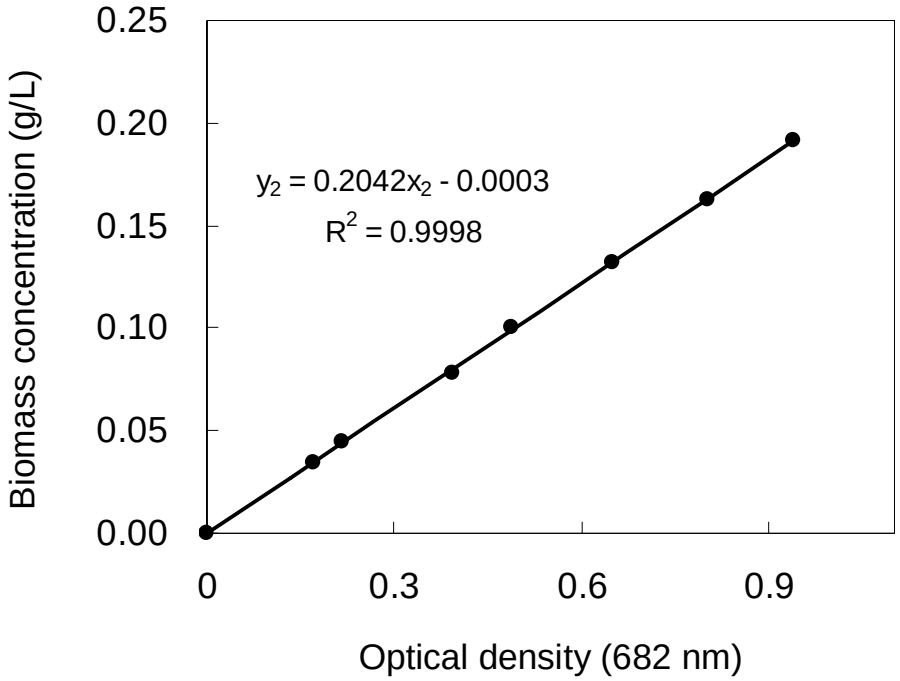


Figure3-8 Calibration curves and equations of optical density of *Nannochloropsis oculata* at A_{682} to the biomass concentration (g/L). The calibration curve, correlative equation and R^2 value of each correlation were indicated.

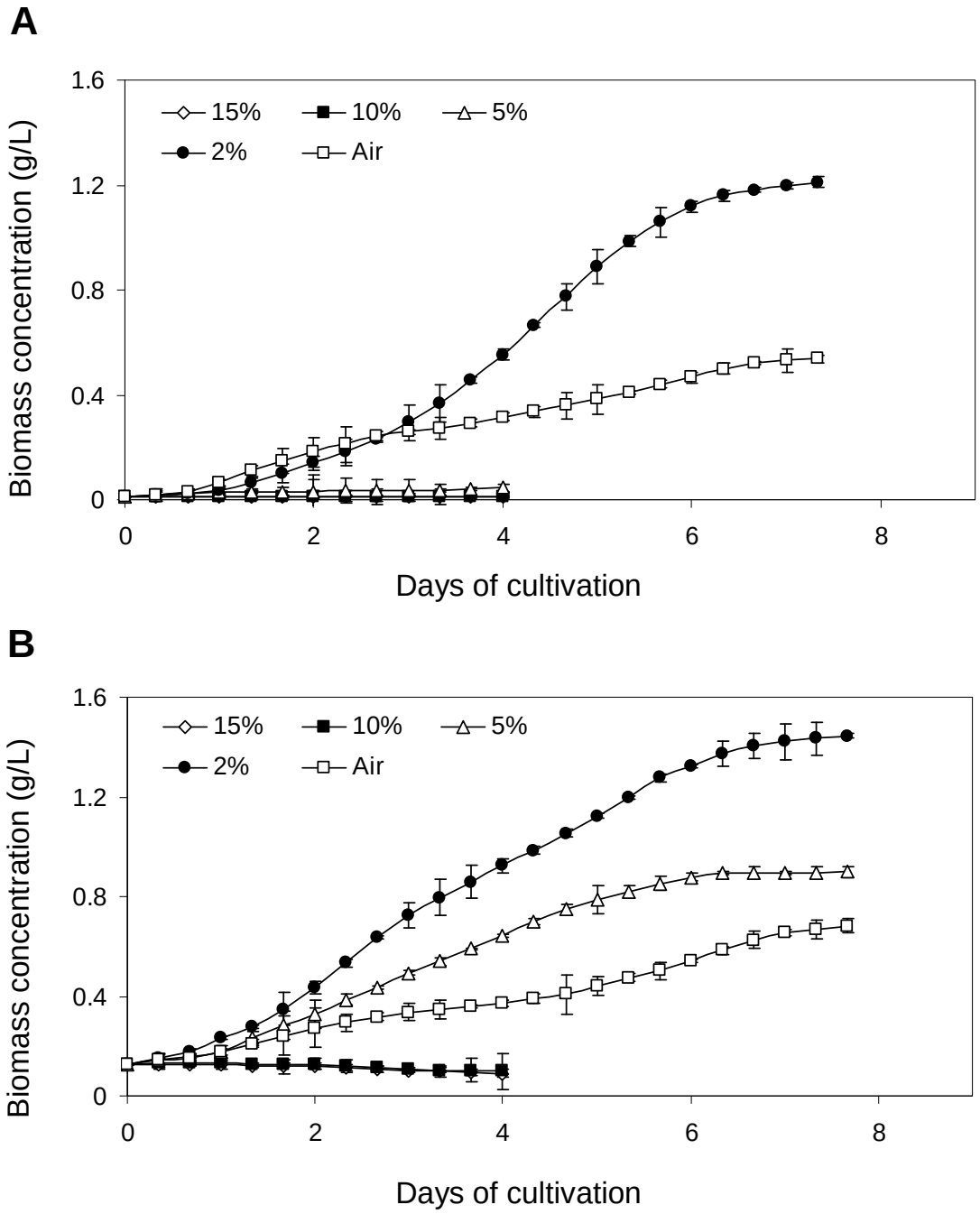


Figure 3-9. Effects of different concentrations of CO_2 aeration on the growth of *Chlorella* sp.. **A**, shows the growth curve of *Chlorella* sp. inoculated at low-density cells (8×10^5 cells/mL in an 800-mL cultivation). **B**, shows the growth curve of *Chlorella* sp. inoculated at high-density cells (8×10^6 cells/mL in an 800-mL cultivation). All experiments were carried out in triplicate. The cultures were illuminated 24 hr at 300 μmol/m^2/s and bubbled with a flow rate of 0.25 vvm airstreams at 26 ± 1°C.

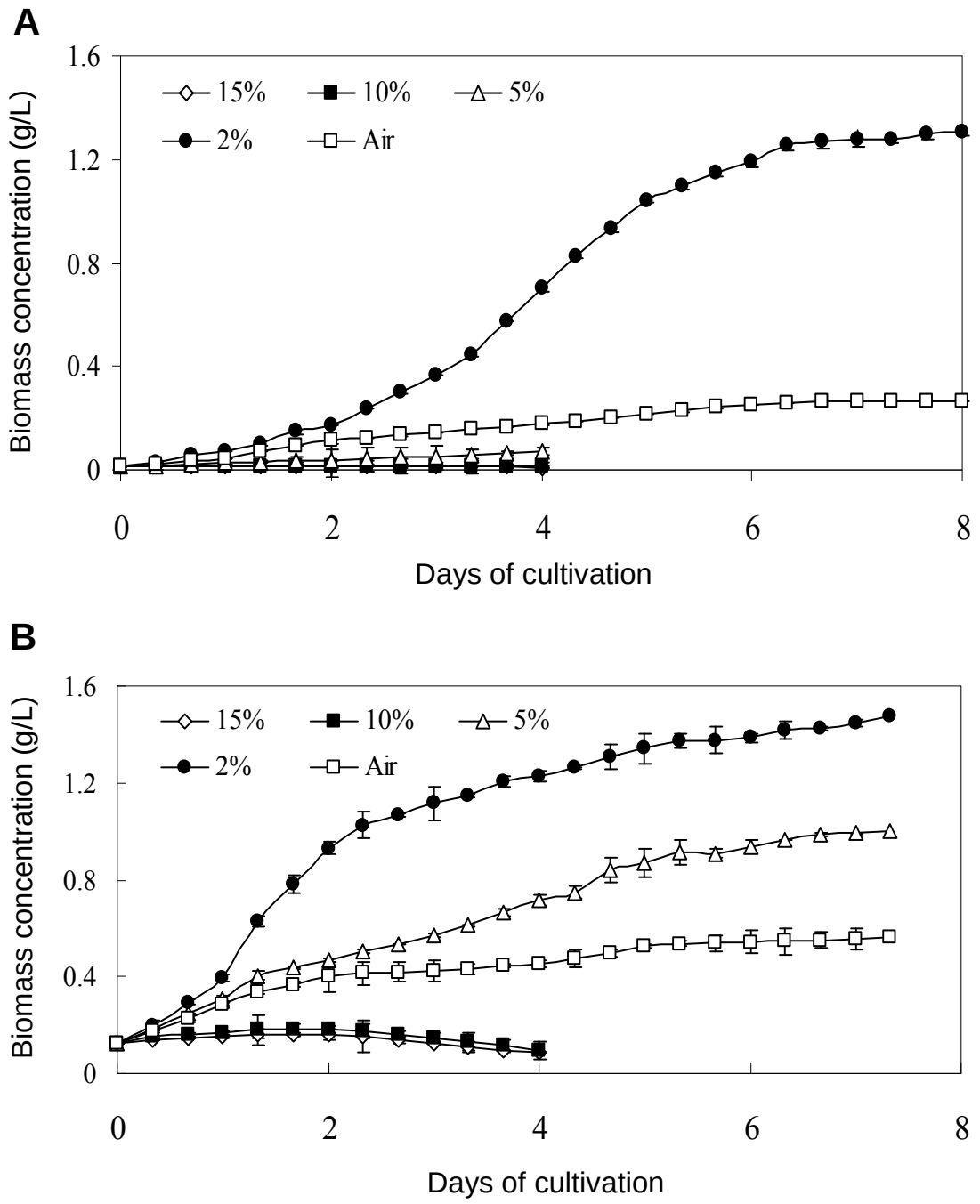


Figure 3-10. Effects of different concentrations of CO_2 aeration on the growth of *Nannochloropsis oculata*. **A**, shows the growth curve of *Nannochloropsis oculata* inoculated at low-density cells (8×10^5 cells/mL in an 800-mL cultivation). **B**, shows the growth curve of *Nannochloropsis oculata* inoculated at high-density cells (8×10^6 cells/mL in an 800-mL cultivation). All experiments were carried out in triplicate. The cultures were illuminated 24 hr at 300 μmol/m^2/s and bubbled with a flow rate of 0.25 vvm airstreams at 26 ± 1°C.

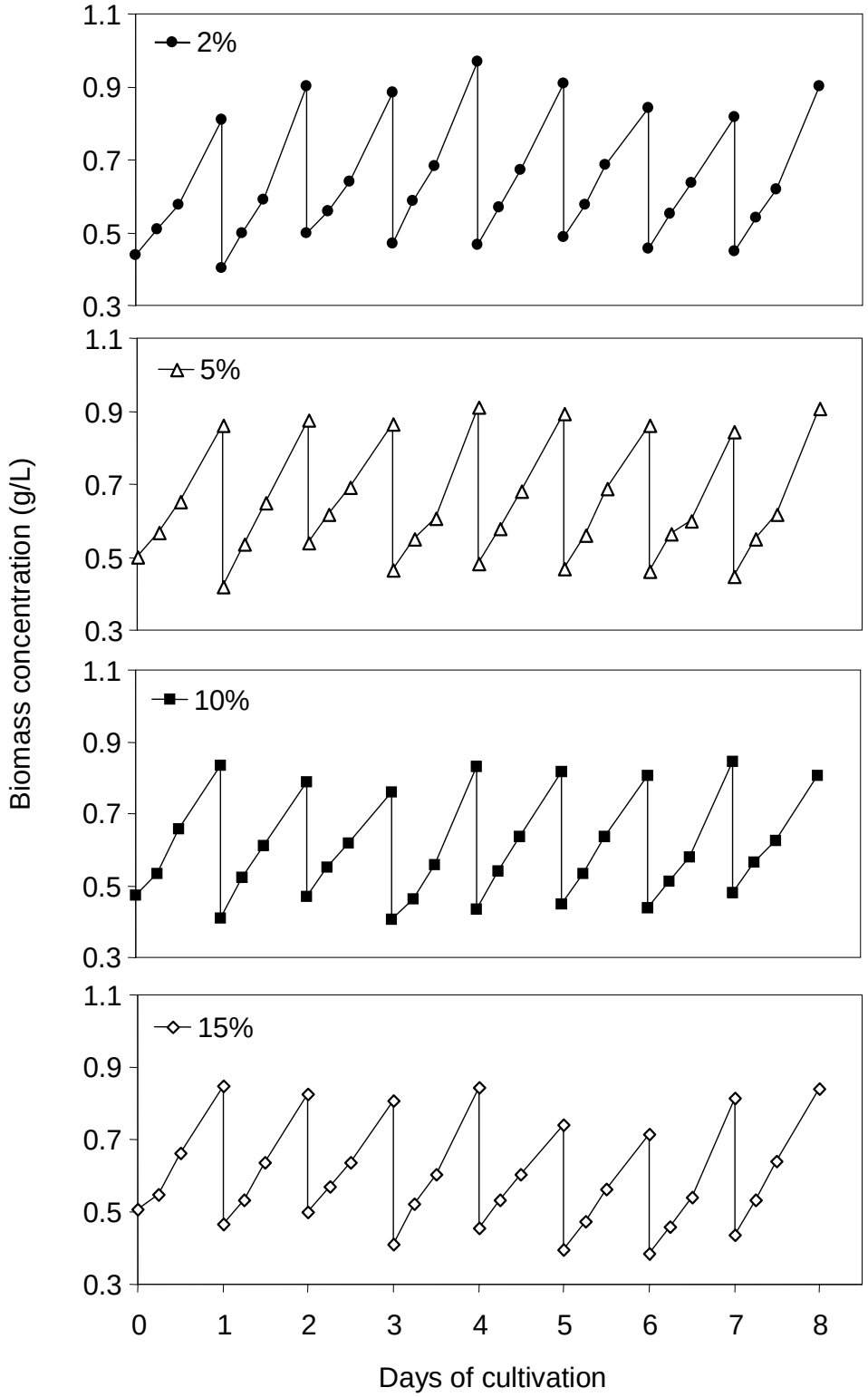


Figure 3-11. Growth profiles of *Chlorella* sp. cultured in the semi-continuous system aerated with 2, 5, 10, and 15% CO_2. In the cultures, approximate 0.4 g/L of microalgal cells was inoculated and cultivated under 24 hr illumination at 300 μmol/m^2/s and bubbled with a flow rate of 0.25 vvm airstreams at 26 ± 1°C for 8 d. Amount of 50% of culture broth was replaced with the fresh modified f/2 medium at interval of 24 hr.

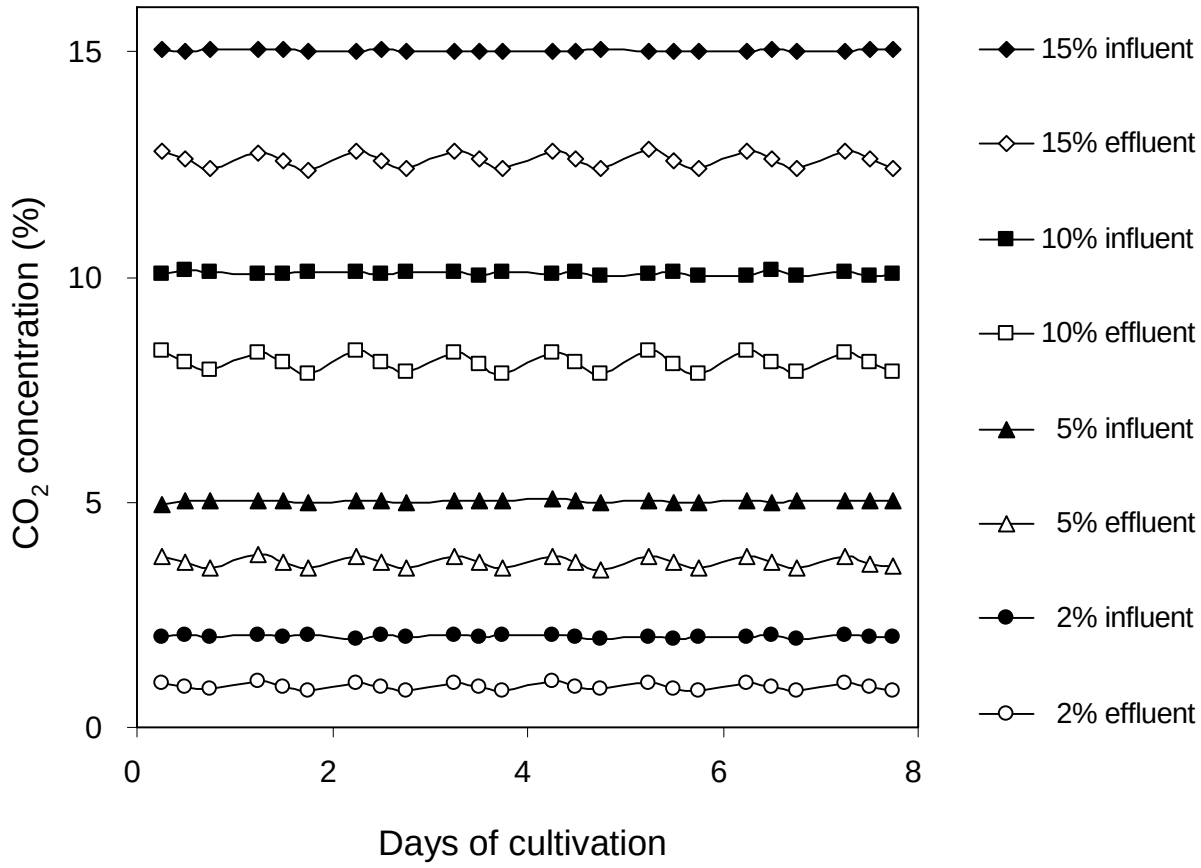


Figure 3-12. Influent *vs*. effluent CO_2 loading in airstreams during the operation of single semi-continuous *Chlorella* sp. cultures under 2%, 5%, 10%, and 15% CO_2 aeration. Amount of 50% of culture broth was replaced with the fresh modified f/2 medium at interval of 24 hr. The influent and effluent CO_2 concentrations in each culture were measured at 6, 12, and 18 hr after the cultured broth was replaced each day. The microalgal cells was cultivated under 300 μmol/m^2/s for 24 hr lighting, at 26 ± 1°C and the gas provided as a flow rate of 0.25 vvm airstreams. The average influent and effluent CO_2 concentrations of each day were then calculated.

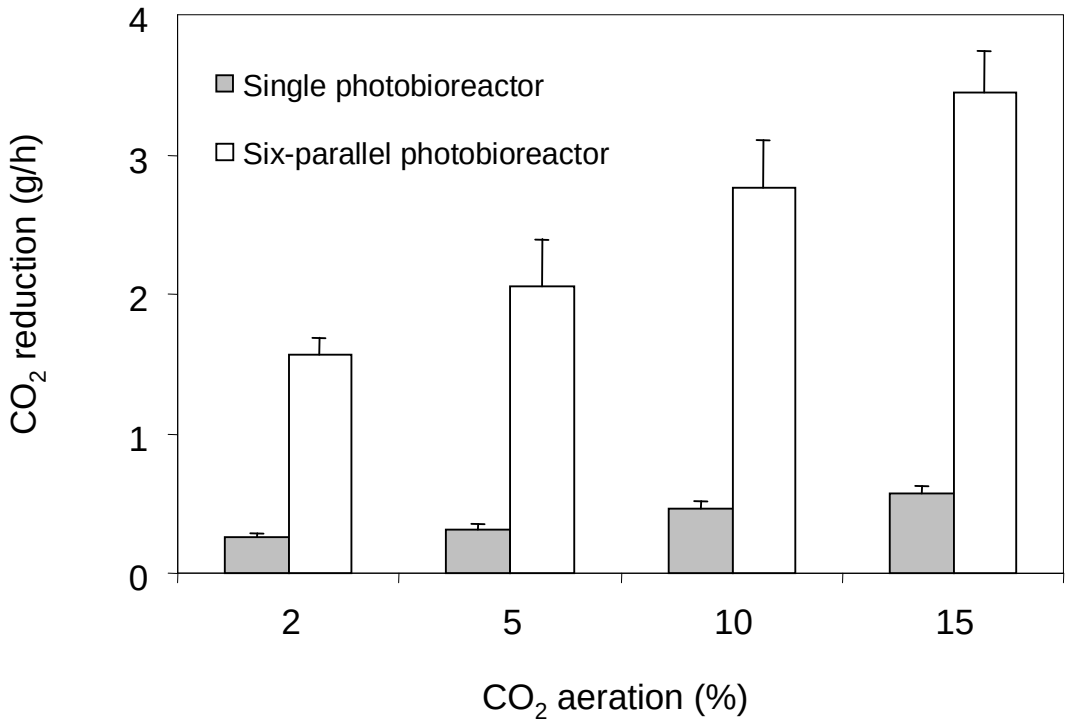


Figure 3-13. Comparisons of the total amount CO_2 reduction in the single and six-parallel photobioreactor of semi-continuous *Chlorella* sp. cultures under 2%, 5%, 10%, and 15% CO_2 aeration with a flow rate of 0.25 vvm. The figure shows the total amount of CO_2 reduction by single and six-parallel photobioreactor. The total amount of CO_2 reduction was determined by the difference of influent and effluent CO_2 loading in airstreams. Each data indicates the mean ± SD, were measured from three independent cultures.

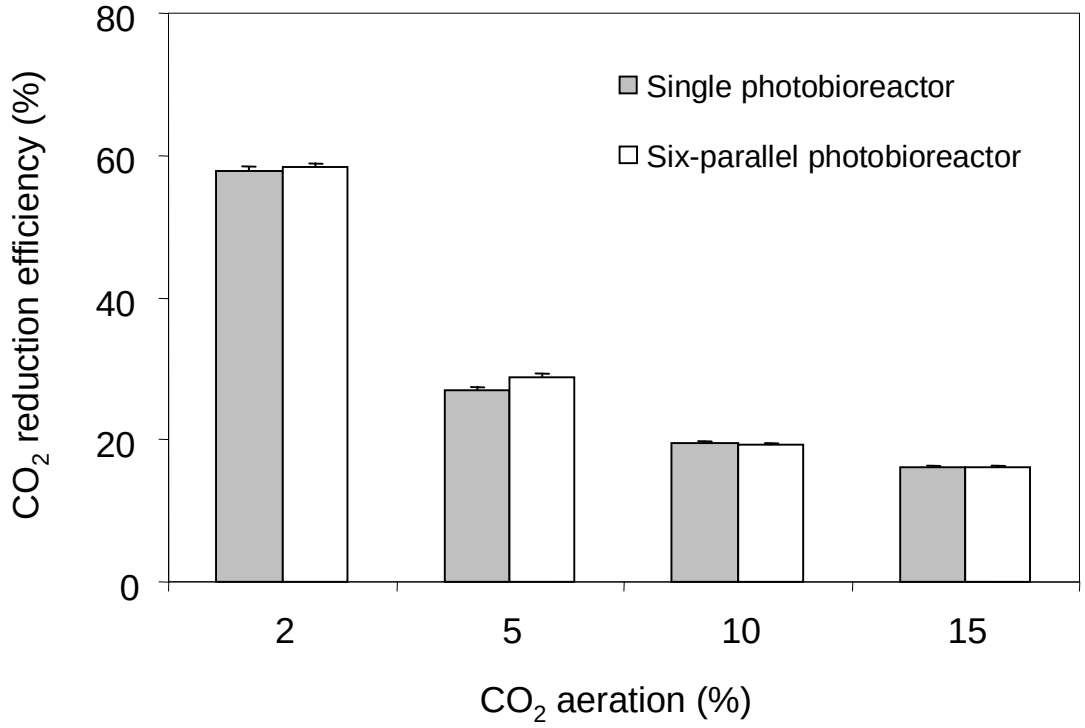


Figure 3-14. Comparisons of the CO_2 reduction efficiency in the single and six-parallel photobioreactor of semicontinuous *Chlorella* sp. cultures under 2%, 5%, 10%, and 15% CO_2 aeration with a flow rate of 0.25 vvm. The figure shows the efficiency of CO_2 reduction by single and six-parallel photobioreactor. The CO_2 reduction efficiency was determined by the difference of influent and effluent CO_2 loading in airstreams. Each data indicates the mean ± SD, were measured from three independent cultures.

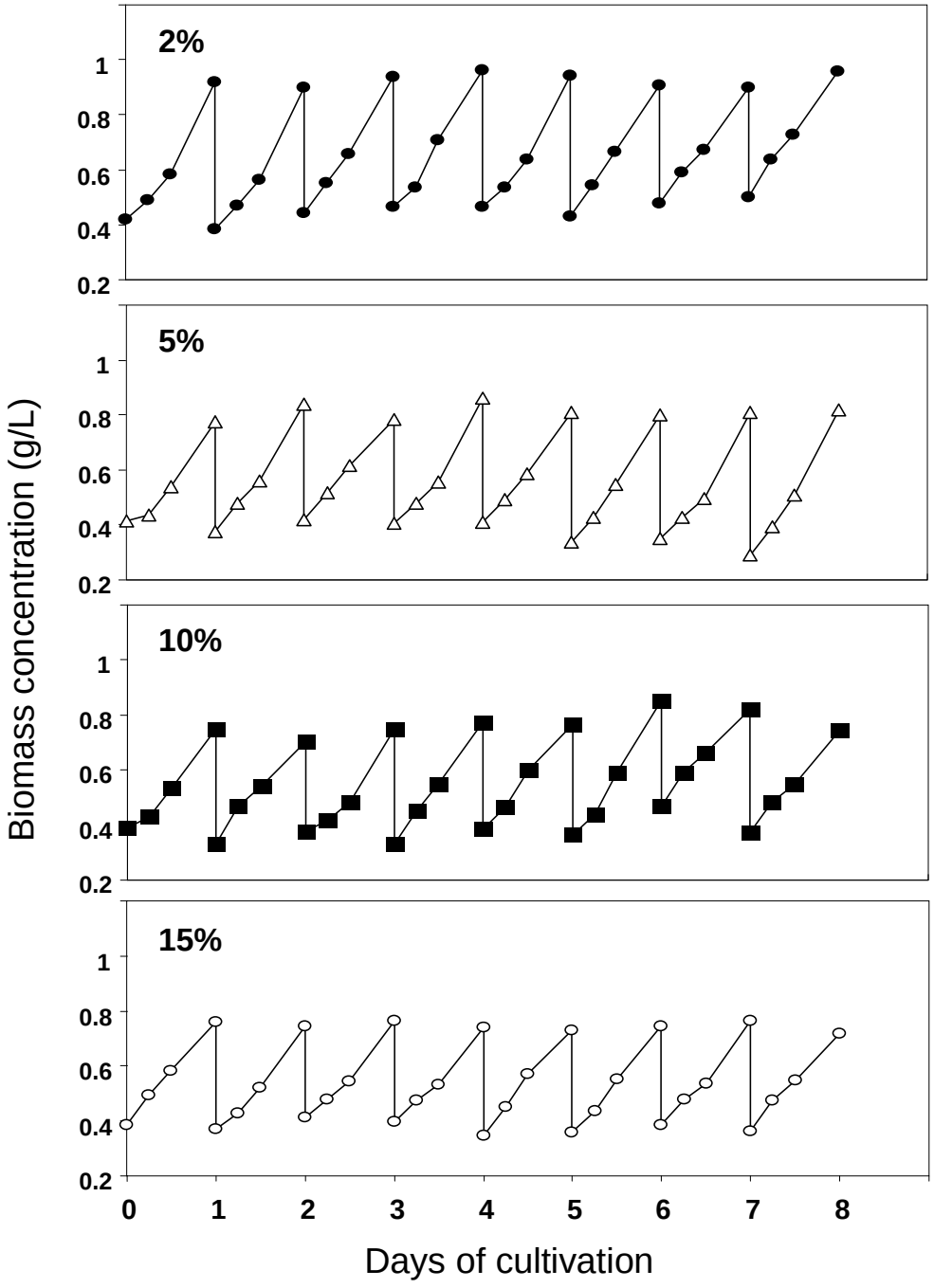


Figure 3-15. Growth profiles of *Nannochloropsis oculata* cultured in the semi-continuous system aerated with 2, 5, 10, and 15% CO_2. In the cultures, approximate 0.4 g/L of microalgal cells was inoculated and cultivated under an illumination at 300 μmol/m^2/s for 24hr lighting and bubbled with a flow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

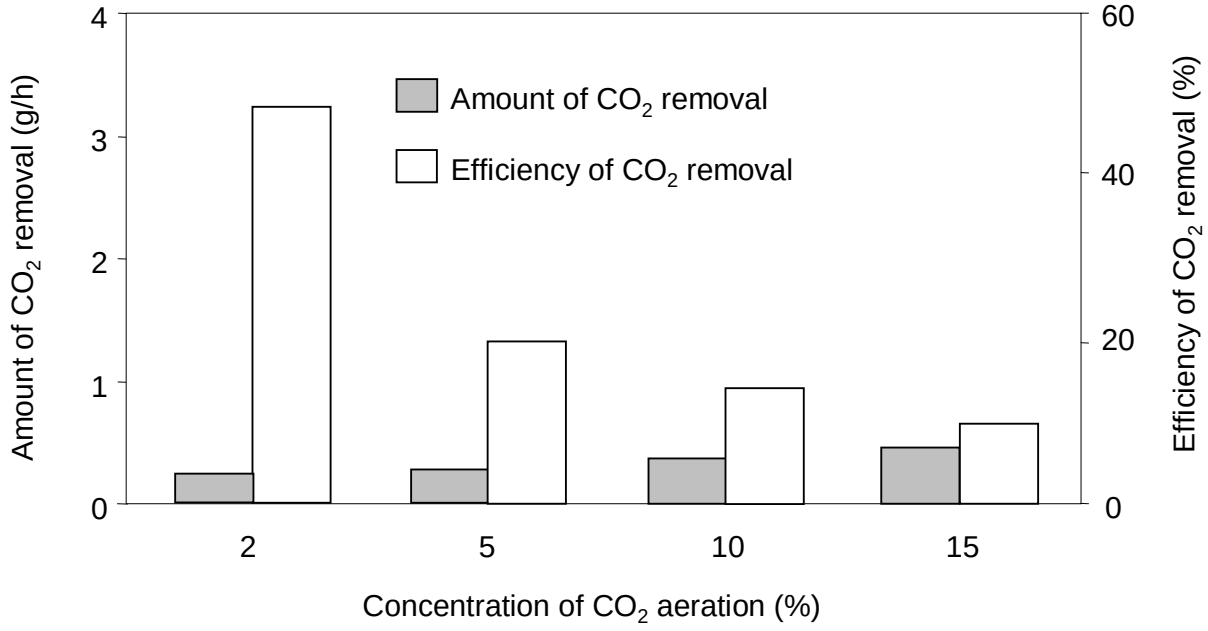
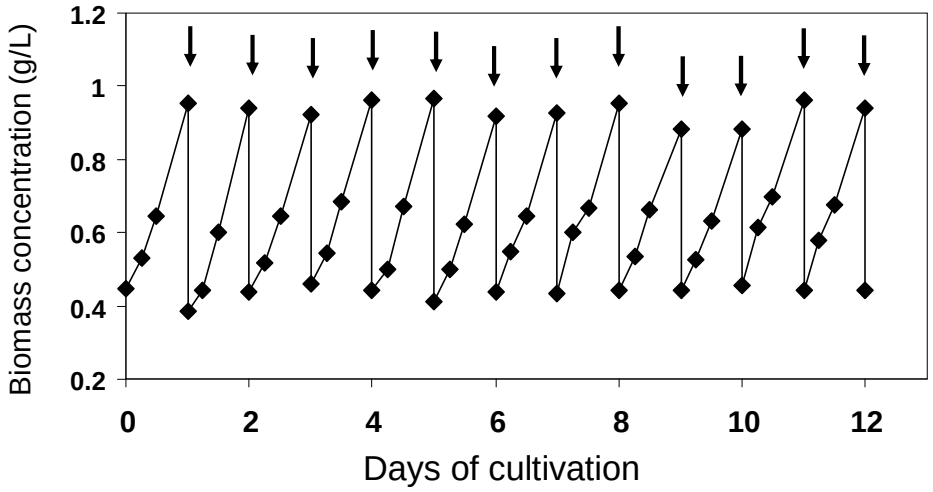


Figure 3-16. CO_2 removal in the airstreams during the operation of semi-continuous *Nannochloropsis oculata* cultures under 2, 5, 10, and 15% CO_2 aeration with a flow rate of 0.25 vvm. The total amount and efficiency of CO_2 reduction were determined by the difference of influent and effluent CO_2 loading in airstreams. The influent and effluent CO_2 concentrations in each culture were measured at 24 hr after the cultured broth was replaced each day. The average influent and effluent CO_2 concentrations of each day were calculated.

A. One-day replacement



B. Three-day replacement

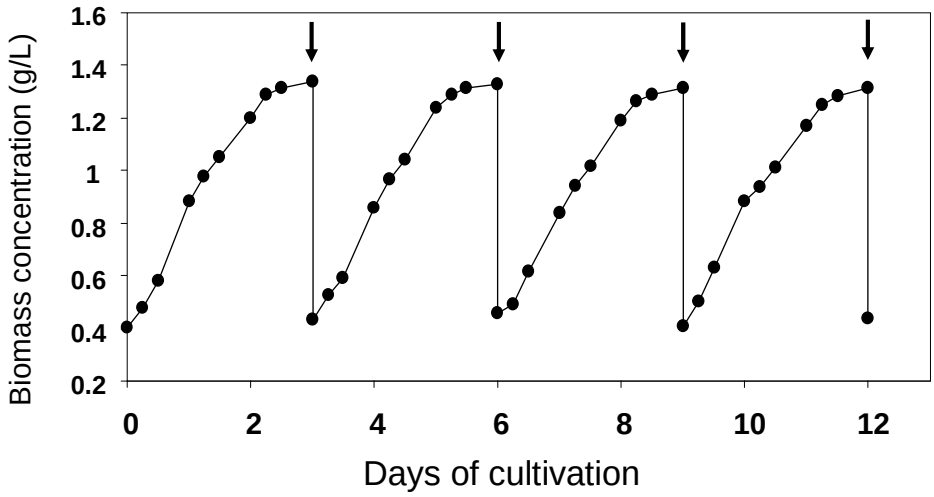


Figure 3-17. Growth profiles of *Nannochloropsis oculata* cultured in the semi-continuous system with 2% CO_2 aeration and operated by one-day replacements (**A**) and three-day replacements (**B**). In the cultures, approximate 0.4 g/L of microalgal cells was inoculated and cultivated under an illumination at 300 μmol/m^2/s and bubbled with a flow rate of 0.25 vvm airstreams at 26 ± 1°C. 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 hr (one-day replacement; half broth was replaced each day) and 72 hr (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.