國 立 交 通 大 學

生 物 科 技 學 院

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碩 士 論 文

利用工業煙道廢氣培養微藻對其生物質與油脂產量之最佳化探討

Optimizations of microalgae cultivated with the flue gas from steel plant on micaroalgal biomass and lipid productivity

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中 華 民 國 101 年 7 月

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眨眼間，兩年就過去了。從剛開始進入實驗室懵懵懂懂的菜鳥，變成一個在內可以對著自己專業領域侃侃而談的準碩士生，在外可以抬反應器、搬藻桶，甚至連鐵架的扛得起來的壯漢。相信這一段充滿陽光、汗水以及屎臭味的光陰，讓原本的我更加成熟，更有自信的面對未來的挑戰。這段求學路途中，最要感謝的就是我的指導老師林志生教授，在您嚴厲的教導下，總是時時刻刻透露出對我們未來的關心，害怕我們將來出社會沒有競爭力，所有教誨和叮嚀皆因恨鐵不成鋼，謝謝老師的指導，在這裡獻上最誠摯的感謝。感謝這次的口委老師曾慶平教授和鍾竺均院長，因為你們而讓這場口試蓬蓽生輝，給我很多十分受用的建議，謝謝你們的指導。

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國立交通大學 生物科技學系碩士班

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利用工業煙道廢氣培養微藻對其生物質與油脂產量之最佳化探討

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摘　　要

近年來，大氣中過量累積的二氧化碳成為全球暖化的主因，其中主要多為工廠所排放含大量二氧化碳之工業廢氣，而微藻養殖則被視為解決此問題的方法之一。微藻可行光合作用，利用光照做為能量，將二氧化碳和水轉化為生物質，達到二氧化碳減量與生產生質能料源的目的。本研究應用本實驗室所篩選出之耐溫、高生長速率，且具高二氧化碳耐受性的微藻株 *Chlorella* sp. TT-1，通入中鋼煙道廢氣做為碳源，進行廢氣養殖微藻之試驗，用以產製微藻生物質，並萃取藻油生產生質柴油。本研究更結合反應曲面法 (response surface methodology, RSM) 進行微藻養殖，找出微藻之最適化培養條件，例如微藻養殖之初始濃度、通入廢氣組成比率及廢氣通氣速率等。

本研究利用不同廢氣比例組成的氣體進行微藻養殖，實驗結果顯示以廢氣稀釋為 25 %的混合氣體進行微藻培養時，可達到最佳的生長效率，其生物質產率可達 0.421 g/L/day；再則，以廢氣比率為 25 %所培養的微藻，其油脂累積量最多，可達約 40%的油脂含量，因此在不同的廢氣組成比例的培養下，微藻的生長速率和產脂效應會有所差異，不同的廢氣組成比率並不會顯著的影響微藻脂肪酸甲酯 (fatty acid methyl ester, FAME) 的組成。另外不同的廢氣通氣速率對微藻的生物質產率會有顯著性的影響，在 0.3 vvm 的通氣速率下微藻有最大的生物質產率，為 0.286 g/L/day；在油脂含量的部分，在通氣速率 0.3 vvm 下的油脂累積可達約 40%；通氣速率對 FAME 的累積也有影響，於 0.3 vvm 之高通氣速率下有最多之 FAME，比低通氣速率下之微藻其 FAME 在微藻內的含量多約 4~5 %。

為探討光照於利用工廠廢氣養殖微藻試驗上之影響，我們將養殖的 *Chlorella* sp.

TT-1 通入工廠廢氣，並以全日照和半日照兩種不同的光照模式進行微藻養殖。結果顯示微藻之生物質產率在全日照和半日照下，分別為 0.299 g/L/day 和 0.137 g/L/day，明顯可得知微藻在以廢氣培養下，全日照會有較高的產率；另外，微藻之油脂含量在全日照和半日照下分別為 37 %和 15 %，可看出油脂的合成累積與光照時間是具有相關性；在 FAME 的部分，以半日照所培養之 *Chlorella* sp. TT-1 的飽和脂肪酸(C16:0)比例較多，而以全日照培養則有較多的不飽和脂肪酸(C18:1 和 C18:2)的累積。

我們根據上述的實驗結果，結合反應曲面法進行微藻養殖最佳化探討，RSM 可做實驗因子設計並模擬反應曲面，以較少的實驗成本和時間獲得可信賴且有效的資訊，並可討論因子間的交互作用，進而探討多因子對實驗結果的影響性，並以實驗結果得一模擬公式，找出最適之操作條件。本研究利用微藻培養初始濃度、廢氣組成比率及廢氣通氣速率為實驗因子，探討微藻培養最適化之條件。實驗結果顯示，當微藻株在初始濃度為 0.37 g/L、通氣量為 0.30 vvm 及工業煙道廢氣比率為 75 %時，可得到最佳的生物質產率 0.486 g/L/day；在初始濃度為 0.35 g/L、通氣量為 0.24 vvm 及工業煙道廢氣比率為 75 %時，可得到最佳的油脂產率 0.216 g/L/day；在初始濃度為 0.37 g/L、通氣量為 0.25 vvm 及工業煙道廢氣比率為 74 %時，可得到最佳的脂肪酸甲酯產率 0.157 g/L/day。此外，在上述實驗所得出之最佳微藻養殖參數下，我們另外再增加光照強度至 500 μmol/m^2/s 和 700 μmol/m^2/s，以探討其對生長速率和產脂效應的影響。在 500 μmol/m^2/s 下微藻有最大的生物質產率 0.390 g/L/day，且油脂累積可高達約 49 %，另外在 FAME 的組成部分，500 μmol/m^2/s 可顯著促進 C16:0 脂肪酸的累積。

Optimizations of microalgae cultivated with the flue gas from steel plant on micaroalgal biomass and lipid productivity

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Abstract

In the recent years, global warming becomes more serious problem due to the increasing carbon dioxide (CO_2) accumulated in the atmosphere, and the plant steel for industry plays the important role in emitting flue gas which is CO_2-rich. Microalgae are the candidate to solve the problem by photosynthesis, which use sun light as energy source to convert water and CO_2 into biomass, and it can reduce the CO_2 emission and produce biomass. We utilized the isolated thermal- and CO_2-tolerant mutant microalga *Chlorella* sp. TT-1 to reduce CO_2 in flue gas from the steel plant and produce microalgal biomass which can be extracted oil to produce biodiesel. Furthermore, we cultivated the microalgae combined with response surface methodology to get the optimized cultivation conditions under the specific initial density, aeration rate, and flue gas ratio.

To investigate the effect of flue gas ratio, there were different flue gas ratio gases utilized to study the effect of flue gas ratio in microalgal cultivation. The microalga *Chlorella* sp. TT-1 aerated with 25 % flue gas had more biomass productivity, which was 0.421 g/L/day. Lipid content of *Chlorella* sp. TT-1 cultures with 25 % flue gas ratio aeration were 40%. In the part of FAME production, there was no difference between the fatty acid methyl ester (FAME) content of *Chlorella* sp. TT-1 cultivated with different flue gas ratios. In the part about the effect of aeration rate, the biomass productivity of *Chlorella* sp. TT-1 at 0.3 vvm aeration rate has the maximum value, and it is 0.286 g/L/day. The maximum lipid contents of *Chlorella* sp. TT-1 was 40 % when it cultivated with 0.3 vvm aeration rates, and the experimental result was obtained that the biomass production and lipid content in microalga cells increased with the increasing aeration rate. The experimental result about FAME content showed it would

slightly increase with high aeration rate comparing to low aeration rate, and the variance was 4~5 % in total FAME content.

In order to investigate the effect of illumination in microalgae culture aerated with flue gas, *Chlorella* sp. TT-1 was cultivated under full and half illumination. The biomass productivity of *Chlorella* sp. TT-1 in full and half illumination were 0.299 g/L/day and 0.137 g/L/day, respectively. The better irradiation time is full illumination for microalgal cultivation, and the light should be devised when the microalgae cultivated with flue gas. The lipid content of *Chlorella* sp. TT-1 under full and half illumination were approximately 37 % and 15 %, respectively. Obviously, the lipid in microalgal cells under full illumination was more than it cultivated under half illumination. The saturated fatty acid (C16:0) accounted for the most part in *Chlorella* sp. TT-1 cultivated under half illumination, while the microalgae had more long and unsaturated fatty acid (C18:1 and C18:2) under full illumination.

Instead of enormous experiments to test, we cultivated microalga *Chlorella* sp. TT-1 combining response surface methodology which can reduce the experimental times to diminish the cost, and simulate the experimental formula to get the optimum condition for microalgal cultivation. The optimized biomass productivity of *Chlorella* sp. TT-1 is 0.486 g/L/day when the initial density of 0.37 g/L aerated with 75 % flue gas ratio at 0.30 vvm aeration rate; the optimized lipid productivity of *Chlorella* sp. TT-1 is 0.216 g/L when the initial density of 0.35 g/L aerated with 75 % flue gas ratio at 0.24 vvm aeration rate; the optimized FAME productivity of *Chlorella* sp. TT-1 is 0.157 g/L when the initial density of 0.37 g/L aerated with 74 % flue gas ratio at 0.25 vvm aeration rate. In order to investigate whether the enhancement of the illumination is necessary in microalgal culture aerated with flue gas under the optimum condition of microalgal cultivation and the effect of different illumination intensity for microalgal cultivation, the different illuminations were utilized and irradiated for the microalgal cultivation with flue gas. The microalgal cultivation cultivated with 500 μmol/m^2/s had the maximum microalgae biomass productivity 0.390 g/L/day. The illumination of 500 μmol/m^2/s could enhance the lipid accumulation, and it could obtain the lipid content of 49%. Furthermore, the culture irradiated under 500 μmol/m^2/s had more C16:0 content, and it means higher illumination would induce microalgae to synthesize the saturated carbon compound of shorter chain.

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I. Literature Review

1.1 General introduction

The industry revolution from 18th century brought with the rapid economic development, and it improved our living standard. However, there are enormous by-products like greenhouse gas accumulated in the atmosphere. These greenhouse gases absorb and emit radiation at specific wavelengths within the spectrum of thermal infrared radiation emitted by the earth's surface, and this property results in the greenhouse effect. Originally, the greenhouse gases, which include water vapor, carbon dioxide (CO_2), methane (CH_4), nitrous oxide (NO_x), ozone (O_3) and chlorofluorocarbons, play the important roles in maintaining the temperature of the earth, and its character makes the environment of the earth habitable for livings [Karl et al., 2003]. However, more and more greenhouse gases especially CO_2 are emitted, and it makes the heat accumulated in the atmosphere. Global warming, which is induced by increasing concentrations of greenhouse gases in the air, is of great concern [Watanabe et al., 1996]. CO_2 is the principle greenhouse gas, and the global temperature is rapidly rising with the increasing concentration of CO_2. The global atmospheric concentration of CO_2 has increased from 280 ppm of a pre-industrial value to 390 ppm in 2010 [McGin et al., 2011]. This causes the drastic climate change, and it makes a lot of organisms endangered. In addition, energy crisis is another serious problem.

There are various strategies on CO_2 sequestration which have been carried out, such as physical, chemical, and biological methods. There are some examples of physical method like wet absorption or dry adsorption, and geological storage of CO_2, which CO_2 is injected into reservoirs [Granite et al., 2005; Yang et al., 2008; Favre et al., 2009; Ball et al., 2010; Aydin et al., 2010]. However, to use these techniques, purified CO_2 is required, and it is expensive to separate and purify CO_2 from the industrial emissions such as flue gas. It is very necessary and valuable to develop a technique which can directly remove or fix CO_2 from the flue gas. Whereas the examples chemical methods are by washing with alkaline solutions, multiwalled carbon nanotubes, amine coating activated carbon for CO_2 capture in order to reduce the emission of CO_2. The biological method using microalgae photosynthesis is considered as an effective approach for biological CO_2 fixation. By the biological approaches, CO_2 can be fixed into microalgae biomass via photosynthesis. Microalgae has approximately 10~50 times higher CO_2 fixation rates than terrestrial plants and can thus utilize CO_2 from flue gas to

produce biomass. Brennan showed One kilogram of algal dry cell weight utilizes around 1.83 kg of CO_2. Annually around 54.9 ~ 67.7 tones of CO_2 can be sequestered from raceway ponds corresponding to annual dry weight biomass production rate of 30~37 tons per hectare [Brennan et al., 2010]. Therefore, reduction of the emission from industries or power plants by the use of microalgae incorporated photobioreactor is a potential method for removing CO_2 from waste gas.

In addition, microalgae can use CO_2 efficiently and accumulate lipid which is chemically similar to common vegetable oils. Furthermore, it is high potential source for biodiesel production. Instead of fossil fuels, the biodiesel produced from microalgae is more renewable, biodegradable, and low pollutant produced. Thus, reducing atmospheric CO_2 by microalgae photosynthesis is considered safe and reliable for nature.

1.2 Microalgae

Microalgae cover all unicellular and simple multi-cellular microorganisms, including both prokaryotic microalgae and eukaryotic microalgae. In a multistep process of photosynthesis plants and algae (green algae and cyanobacteria) fix CO_2 into sugar using light and water as energy and electron source, respectively. The overall reaction for photosynthesis is given by:

$$CO_2 + H_2O + \text{light} \rightarrow (CH_2O)_n + O_2$$

They can grow almost anywhere, requiring sunlight and some simple nutrients, although the growth rates can be accelerated by the addition of specific nutrients and sufficient aeration.

Microalgae can either be autotrophic or heterotrophic. The former requires only inorganic compound such as CO_2, salts and light energy source for growth while the latter which is non-photosynthetic requires external source of organic compounds for nutrients as energy source. Sometimes microalgae can be autotrophic or heterotrophic when it exists in different condition, and it is called as mixotrophilic. It performs photosynthesis as the main energy source, though both organic compounds and CO_2 are essential. Amphitrophy, subtype of mixotrophy, means that organisms are able to live either autotrophically or heterotrophically, depending on the concentration of organic compounds and light intensity

available. Photoheterotrophycally, also known as photoorganitrophy, photoassimilation, photometabolism, describes the metabolism in which light is required to use organic compounds as carbon source. The photoheterotrophic and mixotrophic metabolisms are not well distinguished, in particular they can be defined according to a difference of the energy source required to perform growth and specific metabolite production.

Microalgae are present in all existing earth ecosystems, not just aquatic but also terrestrial, representing a big variety of species living in a wide range of environmental conditions. It is estimated that more than 50,000 species exist, but only a limited number, of around 30,000, have been studied and analyzed. While among the 10,000 species which are believed to exist, only a few thousand strains are kept in collections, a few hundred are investigated for chemical content and just a handful are cultivated in industrial quantities [Richmond, 2004; Olaizola, 2003]. This collection attests to the large variety of different microalgae available to be selected for use in a broad diversity of applications, such as value added products for pharmaceutical purposes, food crops for human consumption and energy source.

Many research reports and articles described many advantages of using microalgae for biodiesel production in comparison with other available feed stocks, like microalgae are considered to be a very efficient biological system for harvesting solar energy for the production of organic compounds. Microalgae are non-vascular plants, lacking complex reproductive organs, and many species of algae can be induced to produce particularly high concentrations of chosen, commercially valuable compounds, such as proteins, carbohydrates, lipids and pigments. Microalgae are microorganisms that undergo a simple cell division cycle. The farming of microalgae can be grown using sea or brackish water, and microalgae biomass production systems can easily be adapted to various levels of operational or technological skills.

Production of biodiesel and other bio-products from microalgae can be more environmentally sustainable, cost-effective and profitable, if combined with processes such as wastewater and flue gas treatments. In fact various studies demonstrated the use of microalgae for production of valuable products combined with environmental applications. In addition, depending on the microalgae species various high-value chemical compounds may be extracted such as pigments, antioxidants, β-carotenes, polysaccharides, triglycerides, fatty acids, vitamins, and biomass, which are largely used as bulk commodities in different

industrial sectors. These materials are utilized in pharmaceuticals, cosmetics, nutraceuticals, functional foods, and biofuels fields, and the application of microalgae in producer country are listed in **Table 1-1** [Lorenz et al., 2000; Hejazi et al., 2004; Pulz et al., 2004; Ratledge, 2004; Spolaore et al., 2006a; Loubiere et al., 2009]. For examples, some microalgae have been exploited for millennia (*Nostoc* in China and *Arthrospira* in Chad and Mexico). Currently, they have several applications from human and animal nutrition to cosmetics and the production of high-value molecules (fatty acids, pigments, and stable isotope biochemicals). Recently the utilization of microalgae can serve enormous purposes which are potential and feasible. Some possibilities currently being considered are listed below [Mata et al., 2010].

- Microalgae can provide feedstock for several different types of renewable fuels such as biodiesel, methane, hydrogen, ethanol, among others. Algae biodiesel contains no sulfur and performs as well as petroleum diesel, while reducing emissions of particulate matter, CO, hydrocarbons, and SO_x. However emissions of NO_x may be higher in some engine types [Delucchi, 2008].
- Removal of CO_2 from industrial flue gases by microalgae bio-fixation, reducing the GHG emissions of a company or process while producing biodiesel [Wang et al., 2008].
- Wastewater treatment by removal of NH_4^+, NO_3^-, PO_4^{3-}, making algae to grow using these water contaminants as nutrients [Wang et al., 2008].
- After oil extraction the resulting algae biomass can be processed into ethanol, methane, livestock feed, used as organic fertilizer due to its high N:P ratio, or simply burned for energy cogeneration (electricity and heat) [Wang et al., 2008]
- Combined with their ability to grow under harsher conditions, and their reduced needs for nutrients, they can be grown in areas unsuitable for agricultural purposes independently of the seasonal weather changes, thus not competing for arable land use, and can use wastewaters as the culture medium, not requiring the use of freshwater.
- Depending on the microalgae species other compounds may also be extracted, with valuable applications in different industrial sectors, including a large range of fine chemicals and bulk products, such as fats, polyunsaturated fatty acids, oil, natural dyes, sugars, pigments, antioxidants, high-value bioactive compounds, and other fine

chemicals and biomass [Li et al., 2008a; Li et al., 2008b; Raja et al., 2008].

- Because of this variety of high-value biological derivatives, with many possible commercial applications, microalgae can potentially revolutionize a large number of biotechnology areas including biofuels, cosmetics, pharmaceuticals, nutrition and food additives, aquaculture, and pollution prevention [Rosenberg et al., 2008; Raja et al., 2008].

Heterotrophic and mixotrophic cultivation could be a possible avenue of research. The genetic improvement of microalgae strains is also a present challenge. The use of transgenic microalgae for commercial applications has not yet been reported but holds significant promise. Modified strains could overproduce traditional or newly discovered algae compounds and also serve to express specific genes that cannot be expressed in yeast. This could be of great importance for the production of hydrogen, for example. However, a successful drug discovery is the most promising aspect of microalgae biotechnology because the potential is immense although screening remains limited [Tramper et al., 2003].

1.3 Microalgae cultivation

There are several factors influencing algal growth abiotic factors such as light (quality, quantity), temperature, nutrient concentration, O_2, CO_2 , pH, salinity, and toxic chemicals; biotic factors such as pathogens (bacteria, fungi, and viruses) and competition by other algae; operational factors such as shear produced by mixing, dilution rate, depth, harvest frequency, and addition of bicarbonate.

1.3.1 Temperature

Temperature is the most important limiting factor, after light, for culturing algae in both closed and open outdoor systems, and it even regulates cellular, morphological and physiological responses of microalgae. Higher temperatures generally accelerate the metabolic rates of microalgae, whereas low temperatures lead to inhibition of microalgae growth [Munoz et al., 2006]. The optimal temperature varies among microalgae species. Whereas, Many microalgae can easily tolerate temperatures up to 15 ℃ lower than their optimal, but exceeding the optimum temperature by only 2~4 ℃ may result in the total

culture loss. Furthermore, overheating problems may occur in closed culture systems during some hot days, where the temperature inside the reactor may reach 55 ℃. In the application of removing the CO_2 and other oxides in flue gas emitted from power plants, temperature of flue gas and other sources are around 120 ℃. Feasibility of sequestering CO_2 from flue gas depends on either installing heat exchanger system or using thermophilic species. Several species have been identified which can tolerate high temperature up to 60 ℃ (**Table 1-2**).

1.3.2 Illumination

Sunlight is the most common source of energy for the CO_2 fixation and biomass production optimum of microalgae, to an extent that is rather species-dependent. The light intensity would be the limiting factor if the irradiation does not achieve the level to carry out photosynthesis, and microalgae productivity becomes proportional to the light conversion efficiency [Richmond et al., 2003]. Light intensity requirements of typical microalgae are relatively low compared with those of higher plants, microalgae activity usually rises, with increasing light intensity, up to 400 μmol/m^2/s. However, a strong species-dependence exists that should be taken into account [Munoz et al., 2006; Kumar et al., 2010]; as an example, the saturating light intensity of *Chlorella* and *Scenedesmus* sp. is of the order of 200 μmol/m^2/s [Hanagata, 1992]. While exposure of cells to long period with high light intensity causes photoinhibition due to damage of repair mechanism of photosystem II leading to inactivation of other systems including the oxygen evolving systems, electron carriers and the associated D1/D2 proteins [Rubio et al., 2003]. Whereas, Light above a saturation point causes light inhibition, which can be counterbalanced by exposing microalgae cells to very short cyclic periods of light and darkness [Pulz, 2001]. The ratio of light to dark (or low-intensity light) periods in a cycle is crucial for microalgae productivity [Munoz et al., 2006]. When the light/dark cycle period approaches the photosynthetic unit turnover time (equal to the dark reaction time, estimated to lie within 1~15 ms), maximum photosynthetic efficiencies can be achieved [Richmond et al., 2003]. Furthermore, periods of low light intensity significantly increase growth, CO_2 assimilation and lipid productivity in microalgae for a given illumination compared with periodic darkness. This type of irradiation design can be achieved via artificial light, such as hybrid lighting systems [Chen et al., 2011].

The spatial distribution of the light intensity inside the reactor is, apart from the geometry, mainly influenced by light attenuation caused by mutual shading of the cells via

adsorption by the pigments or via scattering by the cells. Some mechanistic formulae for calculating light gradients in liquid particle systems have been published, however, for a small and flat volume element an exponential development depending on biomass concentration can be assumed [Posten, 2009; Cornet et al., 1995]. According to Eq. (1), the light intensity decreases exponentially with distance from a reactor wall as the concentrations of both cell and product increase

$$\frac{I_L}{I_o} = \exp(-\gamma L) \quad (1)$$

Where I_L is the light intensity at depth L, I_o is the original incident intensity, c is the turbidity. Hence, the light intensity tends to decrease rapidly due to the light shading effects arising from increases in the concentrations of both cell and product or from formation of biofilm on the surface of reactor vessels [Chen et al., 2008; Chen et al., 2011]. Because of the problems and limitations associated with conventional light sources, various photobioreactor designs with different illumination strategies have been developed to enhance the microalgae production rate and oil/lipid content [Ma et al., 1999]. Fernandes studied the effect of circular and plan geometry in light penetration. For similar microalgae cell concentrations, circular geometry allows a better light penetration, than the plane geometry allowing a higher volume fraction of the reactor to receive sufficient amounts of light however, plan geometry helps in uniform distribution of light [Fernandes et al., 2010].

Different lamps generate distinct spectra, and different microalgae species possess dissimilar absorption optima; therefore, each individual case should be studied before deciding on the set point of this important operational parameter [Kumar et al., 2010; Suh et al., 2003a]. In the recent years, LED is the new potential choice to get the cheap, durable, reliable, and highly efficient light source. LEDs which have narrow light emission spectra between 20 and 30 nm can be matched with photosynthetic needs which can match different microalgae species for different appropriate spectrum. For instance, the adsorption wavelength of blue LED and red LED are around 450~470 nm and 645~665 nm, respectively [Yeh et al., 2009]. Wang found that the highest specific growth rate and biomass production were obtained by using red LED in the photoautotrophic cultivation of *Spirulina platensis* [Wang et al., 2007].

1.3.3 Aeration rate

Aeration rate plays the important role in both dissolved inorganic carbon and mixing which influence the microalgae cultivation much. The effects of carbon oxide uptake are different under different aeration rates. In addition, different aeration rates will make the different ratios of CO_2 dissolve in medium, and this phenomenon will improve the efficiency to utilize CO_2 as inorganic carbon source for microalgae.

In the perspective of mixing, low mixing rates hamper gaseous mass transfer and might even permit biomass settling. In either case, poor mixing leads to emergence of stagnant zones, where light and nutrients are insufficiently available and anoxic/anaerobic conditions will thus prevail, which results in a decrease of productivity. Culture viability might also be compromised by production and accumulation of toxic compounds in stagnant zones [Becker, 1994]. However, High liquid velocities and high degrees of turbulence in photobioreactors can damage microalgae due to shear stress, and shear damage is sometimes used as an argument against mechanical mixing in microalgae cultures [Barbosa et al., 2003; Vega-Estrada et al., 2005; Carlsson et al., 2007]. Furthermore, Kaewpintong reported a better growth for an airlift bioreactor than for an unaerated column. This is also explained because aeration lead to a better mixing of the microalgae culture, which prevents sedimentation, maintains homogeneous conditions, and helps for a better contact between cells and nutrients [Kaewpintong, 2004].

In the researches of aeration rate for microalgae cultivation, Ryu demonstrated that the high aeration rate resulted in the high cell concentration of *Chlorella sp.* AG10002 and the low CO_2 utilization efficiency. The optimum aeration rate in consideration of both the productivity and the CO_2 utilization efficiency was 0.20 vvm [Ryu et al., 2009]. Also, Chiu investigated that significant higher of CO_2 fixation rate was also found when the aeration rate decreases in the result of the increasing of CO_2 absorption from bubbling gas. This was caused by the increase of surface area per unit gas volume of the bubble which would also enhance the CO_2 removal efficiency [Chiu et al., 2009b].

1.3.4 Culture pH

Most microalgae species are favored by neutral pH, and have a narrow optimal range of pH. The pH of the culture medium is influenced by dissolving CO_2 concentration and even NO_x and SO_x from the flue gas. With elevated CO_2 concentrations, pH drops down to pH = 5,

and with higher SO_x concentrations even down to pH = 2.6 have been reported [Maeda et al., 1995]. Some species are tolerant to higher pH (e.g. *Spirulina platensis* at pH = 9 [Hu et al., 1998b]) or lower pH (e.g. *Chlorococcum littorale* at pH = 4 [Kumar et al., 2010]). There is a complex relationship between CO_2 concentration and pH in microalgae bioreactor systems, owing to the underlying chemical equilibrium among such chemical species as CO_2, H_2CO_3, HCO_3^- and CO_3^{2-}, and each microalgae strain is appropriate to assimilate different inorganic compound.

1.3.5 Culture density

Both productivity and light utilization efficiency value are the functions of the cell density. It is crucial to select the optimum cell concentration for the efficient CO_2 sequestration. Below the optimum cell concentration, not all the light energy is captured by the cells while at above the optimum cell concentration, a larger proportion of the cell are in the dark due to self-shading which leads the productivity declining due to mainly respiration [Zhang et al., 2001; Kumar et al., 2010]. Light intensity decreases deeper within the culture medium, especially in high-density cultures; hence, the issue of optical depth, which measures the proportion of radiation absorbed or scattered along a path through a partially transparent medium, should be considered in microalgae bioreactor design. The relationship between biomass productivity and cell concentration has been reported in flat inclined modular and vertical flat plate photobioreactor by Hu and Zhang, respectively [Hu et al., 1996; Zhang et al., 2001]. However, highly dense culture also makes cells more tolerant to high percentage of CO_2 concentration [Chiu et al., 2008].

1.3.6 CO_2 concentration

In addition to light and water, carbon dioxide (CO_2) is critical for photosynthesis in higher plants and microalgae [Li et al., 2008a; Tredici, 2010; Chisti, 2007]. Atmospheric CO_2 levels (0.0387 %) are not sufficient to support the high microalgae growth rates and productivities needed for large-scale biofuel production. In principle, flue gas from combustion process typically contains above 15 % CO_2, and this value indicates that flue gas will supply enough carbon sources for large-scale microalgae production. Whereas, algae cells can tolerate CO_2 only up to a certain level after which it becomes detrimental for the growth of the cells because of the two reasons. Firstly environmental stress induced by the higher CO_2 concentration which causes biological reduction in the capacity of algal cells for

CO_2 sequestration [Sobczuk et al., 2000; Kumar et al., 2010]. Secondly at higher CO_2 concentration, the culture pH decreases due to the formation of high amount of bicarbonate buffer (which is described in the part of **1-3-4 pH**). The biomass productivity increases with increase in CO_2 % in the gas mixture up to certain percentage beyond which productivity decreases. CO_2 sequestration experiment operated by Chiu at a flow rate of 0.25 vvm reports that 2 % of CO_2 is optimum for the growth of *Chlorella* while at 10 % specific growth rate becomes insignificant [Chiu et al., 2008]. However, the experiment conducted by Maeda for the sequestration of CO_2 from flue gas emitted by coal fired thermal power plant confirms that *Chlorella* sp. T-1 can tolerate up to 100 % CO_2 concentration but the maximum growth rate was obtained when using 10 % CO_2 with no significant decreasing growth rate up to 50 % CO_2 concentration [Maeda et al., 1995]. They also concluded that pre-adaptation of cells with lower percentage of CO_2 concentration leads the tolerability of cells in higher percentage of CO_2 [Kumar et al., 2011].

1.3.7 O_2 accumulation

Photosynthesis is a reversible set of reactions, and excessive dissolved oxygen, DO (>35 mg/L), can inhibit the metabolic processes [Carvalho et al., 2006]. The water splitting activity of photosystem II is responsible for the oxygen evolution during photosynthesis. Trapped oxygen in the liquid culture causes toxic effects like photo-bleaching and reduces the photosynthetic efficiency. Furthermore, microalgae are negatively charged on their surface, so they can strongly adsorb polyvalent cations; this ion exchange capacity is the basis of the microalgae potential to remove heavy metals from wastewaters [Munoz et al., 2006]. However, heavy metals are potent inhibitors of microalgae photosynthesis because they can replace or block the prosthetic metal atoms in the active site of relevant enzymes, or otherwise induce morphological changes in the microalgae cells that lead to physiological incompatibility [Kumar et al., 2010].

An efficient degassing system is required in order to remove formed O_2. Accumulation of O_2 is a serious problem in reactors with poor gas exchange like horizontal tubular reactors, especially when continuous run tubing increases [Miron et al., 1999]. The problem of accumulation of O_2 increases when a helical tubular reactor is scaled-up by increasing the light harvesting unit. Hence it is necessary to have a separate degassing unit in which the distance between the entrance and exit is such that even smallest bubbles can disengage. To prevent inhibition and damage, the maximum tolerable dissolved oxygen level should not

generally exceed about 400 % of air saturation value [Chisti, 2007]. It is not of major concern in reactors which have an open gas transfer area as in stirred tank and vertical reactors [Kumar et al., 2011].

1.3.8 Nutrient

Except for carbon source from organic (sugar or glucose) and inorganic carbon source (CO_2), vitamins, salts and other nutrients like nitrogen and phosphorous are vital for algal growth [Williams, 2002; Suh et al., 2003b]. Growth medium must provide the inorganic elements that constitute the algal cell. Nitrogen is the most important element that is required for microalgae nutrition, as a constituent of both nucleic acids and proteins, and nitrogen is directly associated with the primary metabolism of microalgae [Becker, 1994]. Fast-growing microalgae species prefer ammonium rather than nitrate as a primary nitrogen source [Green et al., 1996]; intermittent nitrate feeding, however, will enhance microalgae growth if a medium that lacks nitrate is used [Jin et al., 2006]. Under partial nitrogen deprivation, microalgae grow at lower rates (as expected), but produce significantly more lipids, which are reserve compounds synthesized under stress conditions, even at the expense of lower productivities [Lardon et al., 2009; Kumar et al., 2010].

Phosphorus is the third most important nutrient for microalgae growth, and should be supplied to significant excess as phosphates because not all phosphorus compounds are bioavailable (e.g. those combined with metalions) [Kumar et al., 2009]. In the case of marine microalgae, seawater supplemented with commercial nitrate and phosphate fertilizers is commonly used for production of microalgae [Green et al., 1996]. Nevertheless, trace species, such as metals (Mg, Ca, Mn, Zn, Cu and Mb) and vitamins, are typically added for effective cultivation [Becker, 1994].

Minimal nutritional requirements can be estimated using the approximate molecular formula of the microalgae biomass, which is $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$. This formula is based on data presented by Grobbelaar [Chisti, 2007]. Nutrients such as phosphorus must be supplied in significant excess because the phosphates added complex with metal ions, therefore, not all the added P is bioavailable. Sea water supplemented with commercial nitrate and phosphate fertilizers and a few other micronutrients is commonly used for growing marine microalgae [Grima et al., 1999; Kumar et al., 2010].

1.4 Response surface methodology

The *Response Surface Methodology* (*RSM*) is a collection of mathematical and statistical techniques useful for the modeling and analysis of problems in which a response of interest is influenced by several variables and the objective is to optimize this response [Montgomery, 2006]. It is important in designing, formulating, developing, and analyzing new scientific studying and products. The RSM is important in designing, formulating, developing, and analyzing new scientific studying and products. It is also efficient in the improvement of existing studies and products. There are many applications of RSM in industrial, biological, clinical, social, food, physical and engineering sciences.

According to Hill and Hunter, RSM method was introduced by G.E.P. Box and K.B. Wilson in 1951 [Bruns et al., 2010]. Box and Wilson suggested to use a first-degree polynomial model to approximate the response variable. They acknowledged that this model is only an approximation, not accurate, but such a model is easy to estimate and apply, even when little is known about the process. Moreover, Mead and Pike stated origin of RSM starts 1930s with use of Response Curves.

The orthogonal design was motivated by Box and Wilson (1951) in the case of the first-order model. For the second-order models, many subject-matter scientists and engineers have a working knowledge of the central composite designs (CCDs) and three-level designs by Box and Behnken (1960). Also, the same research states that another important contribution came from Hartley (1959), who made an effort to create a more economical or small composite design. There are many papers in the literatures about the response surface models. In contrast, 3-level fractional design has limited works. Thus, 3-level fractional design is an open research subject. Fractional factorial experiment design for factor at 3-levels is a helpful resource conducting this kind of design. Many three-level fractional factorial designs and more importantly their alias tables can be found in their study.

The important development of optimal design theory in the field of experimental design emerged following Word World II. Elfving (1952, 1955, 1959), Chernoff (1953), Kiefer (1958, 1959, 1960, 1962), and Kiefer and Wolfowitz were some of the various authors who published their work on optimality. One of the important facts is whether the system contains a maximum or a minimum or a saddle point, which has a wide interest in industry. Therefore, RSM is being increasingly used in the industry. Also, in recent years more emphasis has been

placed by the chemical and processing field for finding regions where there is an improvement in response instead of finding the optimum response. In result, application and development of RSM will continue to be used in many areas in the future [Stephen M, 1974]

There is an example to explain the principle of response surface methodology. The growth of a plant is affected by a certain amount of water x_1 and sunshine x_2, and the plant can grow under any combination of treatment x_1 and x_2. Therefore, water and sunshine can vary continuously. When treatments are from a continuous range of values, then a Response Surface Methodology is useful for developing, improving, and optimizing the response variable. In this case, the plant growth y is the response variable, and it is a function of water and sunshine. It can be expressed as

$$y = f (x_1 , x_2) + e$$

The variables x_1 and x_2 are independent variables where the response y depends on them. The dependent variable y is a function of x_1, x_2, and the experimental error term, denoted as e. The error term e represents any measurement error on the response, as well as other type of variations not counted in f. It is a statistical error that is assumed to distribute normally with zero mean and variance s^2. In most RSM problems, the true response function f is unknown. In order to develop a proper approximation for f, the experimenter usually starts with a low-order polynomial in some small region. If the response can be defined by a linear function of independent variables, then the approximating function is a first-order model. A first-order model with two independent variables can be expressed as

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \varepsilon$$

If there is a curvature in the response surface, then a higher degree polynomial should be used. The approximating function with two variables is called a second-order model:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} x_{11}^2 + \beta_{22} x_{22}^2 + \beta_{12} x_1 x_2 + \varepsilon$$

In general all RSM problems use either one or the mixture of the both of these models. In each model, the levels of each factor are independent of the levels of other factors. In order to get the most efficient result in the approximation of polynomials the proper experimental design must be used to collect data. Once the data are collected, the Method of Least Square is used to estimate the parameters in the polynomials. The response surface analysis is performed by using the fitted surface. The response surface designs are types of designs for

fitting response surface [Bradley, 2007]. Therefore, the objective of studying RSM can be accomplish by

(1) Understanding the topography of the response surface (local maximum, local minimum, ridge lines), and

(2) Finding the region where the optimal response occurs. The goal is to move rapidly and efficiently along a path to get to a maximum or a minimum response so that the response is optimized.

1.4.1 Central composite design

The central composite design was presented by Box and Wilson [Box et al., 1951]. This design consists of the following parts: (1) a full factorial or fractional factorial design; (2) an additional design, often a star design in which experimental points are at a distance from its center; and (3) a central point. **Figure 1-1** illustrates the full central composite design for optimization of two and three variables. Full uniformly routable central composite designs present the following characteristics:

(1) Require an experiment number according to $N = k^2 + 2k + c_p$, where k is the factor number and c_p is the replicate number of the central point;

(2) α-values depend on the number of variables and can be calculated by $\alpha = 2^{(k-p)/4}$. For two, three, and four variables, they are, respectively, 1.41, 1.68, and 2.00;

(3) All factors are studied in five levels ($-\alpha, -1, 0, +1, +\alpha$).

Figure 1-1 shows representations of central composite designs for two- and three-variable optimization, respectively. **Table 1-3** presents the coded values of the experimental matrices for the application of these designs. Many applications of the central composite design in the optimization of analytical procedures can be found in the literature [Bezerra et al., 2008].

1.5 Bio-mitigation of CO_2, NO_x and SO_x in flue gas with microalgae

Microalgae can typically be used to capture CO_2 from three different sources: atmospheric CO_2, CO_2 emission from power plants and industrial processes, and CO_2 from

soluble carbonate [Wang et al., 2008]. Capture of atmospheric CO_2 is probably the most basic method to sink carbon, and relies on the mass transfer from the air to the microalgae in their aquatic growth environments during photosynthesis [Wang et al., 2008]. However, the potential yield from the atmosphere is limited by low CO_2 concentration in air (360 ppm) which makes it economically infeasible [stepan et al., 2002]. In contrast, Flue gases from power plants are responsible for more than 7 % of the total world CO_2 emissions from energy use [Kadam, 1997]. Also, industrial exhaust gases contains up to 15 % CO_2 [Kadam, 2001; Maeda, 1995], even the higher CO_2 concentration of up to 20 % [Bilanovic et al., 2009], providing a CO_2-rich source for microalgae cultivation and a potentially more efficient route for CO_2 bio-fixation, and adaptability of this process for both photobioreactor and raceway pond systems for microalgae production. Therefore, to use a flue gas emission from an industrial process unit as a source of CO_2 for the microalgae growth is introduced to have a great potential to diminish CO_2 and to provide a very promising alternative to current GHG emissions mitigation strategies [Mata et al., 2010]. However, only a small number of algae are tolerant to the high levels of SO_x and NO_x that are present in flue gases. The gases also need to be cooled prior to injection into the growth medium [Brennan et al., 2010]. A number of microalgae species are able to assimilate CO_2 from soluble carbonates such as Na_2CO_3 and $NaHCO_3$ [Wang et al., 2008]. Due to the high salt content and resulting high pH of the medium, it is easier to control invasive species since only a very small number of algae can growth in the extreme conditions [Wang et al., 2008].

The selection of suitable microalgae strains for CO_2 bio-mitigation has significant effect on efficacy and cost competitiveness of the bio-mitigation process. The desirable attributes for high CO_2 fixation include as following: high growth and CO_2 utilization rates; high tolerance of trace constituents of flue gases such as SO_x and NO_x; possibility for valuable by-products and co-products, like biodiesel and biomass for solid fuels; ease of harvesting associated with spontaneous settling or bio-flocculation characteristics; high water temperature tolerance to minimize cost of cooling exhaust flue gases [Brennan et al., 2010]. In these requisitions, the tolerant to NO_x and SO_x is the most important character the application with flue gas, and the mechanisms of metabolizing CO_2, NO_x and SO_x with microalgae are mentioned as following.

1.5.1 The reduction of CO_2 in flue gas with microalgae

Carbon is the major element of microalgae. The carbon content of microalgae can range

between 36 and 65 %. CO_2 can freely diffuse through the plasma membrane (**Figure 1-2**), suggesting that a CO_2 gradient is not built up along the plasmalemma. Rubisco (ribulose 1,5-bisphosphate carboxylase/oxygenase) is the first enzyme in the Calvin cycle that assimilates CO_2 by converting it into 3-phosphoglycerate (3-PGA). Besides this carboxylase activity, rubisco displays oxygenase activity, using oxygen to form 3-PGA and phosphoglycolate. The latter is subsequently oxidatively metabolised via photorespiration, leading to a loss in CO_2 fixation [Van Den Hende 0et al., 2012]. Since microalgae have a low CO_2-binding capacity of rubisco and the oxygenase activity of rubisco depends on the CO_2:O_2 ratio [Graham et al., 2000], an elevated intracellular CO_2 concentration is a prerequisite. Therefore, some microalgae possess carbon concentrating mechanisms (CCMs) that elevate the CO_2 level at the site of rubisco up to 1000-fold over that of the surrounding medium [Price et al., 2008]. Microalgae CCMs involve: (1) cell membrane inorganic carbon transporters/pumps (e.g., HCO^{3-}/Na + symports or ATP-driven uniports), (2) carbonic anhydrases (CA), (3) specialized cellular structures, and (4) calcification [Van Den Hende S et al., 2012].

A number of research findings have investigated the potential of microalgae for biological carbon capture under various conditions. *C. vulgaris* grown on wastewater discharge from a steel plant successfully sequestered 0.624 g/L/day CO_2 [Yun et al., 1997]. Doucha recorded 10~50 % reduction in CO_2 concentration in flue gases using with *Chlorella* sp., with the efficacy decreasing with increasing rate of flue gas injection into microalgae culture [Doucha et al., 2005]. *Chlorella* Strains from hot springs, which also showed to be tolerant to high temperatures up to 42 ℃, for CO_2 fixation from industrial flue gases containing up to 40 % CO_2 [Sakai et al., 1995]. Chang and Yang found that certain species of *Chlorella* could grow in an atmosphere containing CO_2 up to 40 % [Chang et al., 2003]. In addition to the *Chlorella* species used in the CO mitigation, de Morais and Costa using *Spirulina* sp. obtained a maximum daily CO_2 biofixation of 53.29 % for 6 % CO_2 and 45.61 % for 12 % CO_2 in the injected flue gas, with the highest mean fixation rate being 37.9 % for 6 % CO_2. With S. obliquus, de Morais and Costa achieved biofixation rates of 28.08 % and 13.56 % for 6 % and 12 % CO_2, respectively [de Morais et al., 2007a]. De Morais and Costa found the microalgae species S. obliquus and C. kessleri to be capable of growing in media containing up to 18 % CO_2 [de Morais et al., 2007b]. When comparing B. *braunii*, C. *vulgaris* and *Scenedesmus* sp. under flue gas conditions, Yoo found *Scenedesmus* sp. to be the most suitable for CO_2 mitigation due to high rates of biomass production (0.218 g/L/day) [Yoo et

al., 2010]. B. *braunii* and *Scenedesmus* sp. were found to grow better using flue gas as compared to air enhanced with CO_2. This is in line with an earlier study by Brown who found that microalgae can tolerate flue gas very well [Brown, 1996]. The high cost of process technology and lack of price competitiveness of biodiesel extraction from microalgae versus petroleum diesel are key obstacles to commercial exploitation [Chisti, 2008]. Bio-mitigation of CO_2 emissions provides a complementary function that may be exploited to reduce cost and to enable sustained utilization of microalgae as a biofuel resource.

1.5.2 The reduction of NO_x in flue gas with microalgae

After carbon, nitrogen is the most important nutrient for algal production. Whereas the Redfield C:N ratio of microalgae biomass is 6.66 (molar), this ratio varies in practice among species, with reported values ranging between 3 and 17 [Geider et al., 2002]. In general, microalgae can take up nitrogen in several forms: NH_4^+, NO_3^-, NO_2^-, NO and N_2 (**Figure 1-3**). Both nitrate and nitrite reduction is tightly coupled to energy supply from the photosynthetic electron transfer or external organic carbon [Graham et al., 2000]. Brown found that some of the NO dissolved and was available as an N-source for the microalgae [Brown, 1996]. Nagase concluded that little NO was oxidized in the medium before its uptake by algal cells [Nagase et al., 2001]. Since NO is a small nonpolar molecule, they suggested that NO can diffuse through cell membranes. Moreover, they concluded that NO was preferentially used as a source for microalgae growth rather than nitrate. These researchers based these conclusions on the nitrogen balance calculations. The exact mechanisms through which microalgae use NO_x are still to be proven with more accurate techniques such as nitrogen isotopes [Van Den Hende S et al., 2012].

1.5.3 The reduction of SO_x in flue gas with microalgae

Sulphur, an essential component of the amino acids cysteine and methionine, and S-containing thylakoid lipids, is indispensable for microalgae growth [Graham et al., 2000]. As far as it is known at this stage, microalgae acquire sulphur by taking up sulphate into the cytoplasm, for example, by means of high-affinity sulphate transporter systems [Giordano et al., 2005a, b] (**Figure 1-4**). Sulphate is transported into the plastids or, if present in excess, stored in vacuoles. This stabile sulphate ion is reduced via activation by ATP to 5-adenylsulphate (APS), catalyzed by ATP sulphurylase (ATP-S). APS is then reduced by APS reductase to sulphite (SO_3^{2-}). The produced sulphite is further reduced by sulphite

reductase to sulphide (S^{2-}), which is immediately incorporated into cysteine. On the other hand, S-compounds can also be released by microalgae [Giordano et al., 2005a, b]. For example, Emiliana sp. and Phaecystis sp. generate dimethyl sulfio proprionate (DMSP) for use in osmoregulation or as a cryoprotectant in cold regions [Graham et al., 2000]. This can then be converted into volatile dimethyl sulphide (DMS), which is released from the cells and oxidised to dimethyl sulphoxide (DMSO) to form sulphate aerosols. These aerosols promote an increase in cloud cover and an increase in the earth's albedo (fraction of incident radiation reflected back into space). DMS can be 29 % of the total sulphate assimilation of microalgae and can also generate SO_2 via reactions with NO_3 and OH [Giordano et al., 2005a, b].

1.6 Biodiesel

Biodiesel is a derivative of oil crops and biomass which can be used directly in conventional diesel engines [Clark, et al., 2008]. It is a mixture of monoalkyl esters of long chain fatty acids (FAME) derived from a renewable lipid feedstock which are composed by 90~98 % of triglycerides and small amounts of mono and diglycerides, free fatty acids (1~5 %), and residual amounts of phospholipids, phosphatides, carotenes, tocopherols, sulphur compounds, and traces of water, such as algae oil [Demirbas, 2009; Bozbas et al., 2008]. After the extraction processes, the resulting product algae oil can be converted into biodiesel through a process called transesterification. Transesterification is a multiple step chemical reaction between triglycerides and alcohol in the presence of a catalyst, including three reversible steps in series, where triglycerides are converted to diglycerides, then diglycerides are converted to monoglycerides, and monoglycerides are then converted to esters (biodiesel) and glycerol (by-product) [Sharma, et al., 2009]. The overall transesterification reaction is described in **Figure 1-5** where the radicals R1, R2, R3 represent long chain hydrocarbons, known as fatty acids [Mata, 2010]. The composition of fatty acids significantly depends on the different microalgae species, as they can have a significant effect on the characteristics of biodiesel produced. These are composed of saturated and unsaturated fatty acids with 12–22 carbon atoms, some of them of v3 and v6 families. Thomas analyzed the fatty acid compositions of seven fresh water microalgae species showing that all of them synthesized C14:0, C16:0, C18:1, C18:2, and C18:3 fatty acids. This author reported that the relative intensity of other individual fatty acids chains is species specific, e.g. C16:4 and C18:4 in *Ankistrodesmus* sp., C18:4 and C22:6 in *Isochrysis* sp., C16:2, C16:3 and C20:5 in

Nannochloris sp., C16:2,C16:3, and C20:5 in *Nitzschia* sp. [Thomas et al., 1984].

Although the microalgae oil yield is strain-dependent it is generally much greater than other vegetable oil crops, as shown in **Table 1-4** that compares the biodiesel production efficiencies and land use of microalgae and other vegetable oil crops, including the amount of oil content in a dry weight basis and the oil yield per hectare, per year [Hili, 1984; Peterson, 1998; Rathbauer et al., 2002; Zappi, 2003; Callaway, 2004; Vollmann, 2007; Reijnders, 2008; Kheira, 2009].

The conversion of triglycerides or oil to biodiesel can achieve 98 % [Noureddini, 1998] or greater than 98 % [Anderson, 2003] as an alternative fuel for Diesel engines. The results of the biodiesel product should be quite similar to those of conventional. Diesel in its main characteristics or compatible with conventional petroleum diesel, and it can also be blended in any portion with petroleum diesel. The standard specification for biodiesel fuel for B100 is ASTM D6751 [Sarmidi A, 2009]. The level of blending is designated Bxx, where xx indicates the amount of biodiesel in the blend. The physical and fuel properties of biodiesel from microalgae oil, in general, were comparable to those of diesel fuel. The biodiesel from microalgae oil showed a much lower cold filter plugging point of 11℃ in comparison with that of diesel fuel, as shown in **Table 1-5** [Xu et al., 2006]. Basically, Algal biodiesel has several advantages over petroleum diesel in that: it is derived from biomass and therefore is renewable, biodegradable, and quasi-carbon neutral under sustainable production; it is non-toxic and contains reduced levels of particulates, carbon monoxide, soot, hydrocarbons and SO_x [Brennan, 2010]. Another major advantage of algal biodiesel is in reduced CO_2 emissions of up to 78 % compared to emissions from petroleum diesel [Sarmidi A, 2009].

II. Materials and Methods

2.1 Microalgae strain

The microalgae *Chlorella* sp. TT-1 was originally obtained from the collection of Taiwan Fisheries Research Institute (Tung-Kang, Ping-Tung, Taiwan) and isolated in our laboratory by chemical mutagenesis.

The 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].

2.2 Culture medium and chemicals

Chlorella sp. TT-1 was 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℃. Artificial sea water has following composition (per liter): including 29.23 g NaCl (Showa, Tokyo, Japan), 1.105 g KCl (Showa), 11.0862 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), 0.25 g $NaHCO_3$ (Amresco). f/2 medium has following composition (per liter): 75 mg $NaNO_3$ (Showa), 5 mg $NaH_2PO_4 \cdot H_2O$ (Sigma, Saint Louis, MO, USA), 1 mL of trace metal solution, and 1 mL of vitamin solution [Guillard, 1975]. Trace elemental solution (per liter) includes 4.36 g $Na_2 \cdot$EDTA (Amresco), 3.16 g $FeCl_3 \cdot 6H_2O$ (Sigma), 180 mg $MnCl_2 \cdot 4H_2O$ (Sigma), 10 mg $CoCl_2 \cdot 6H_2O$ (Sigma), 10 mg $CuSO_4 \cdot 5H_2O$ (Sigma), 23 mg $ZnSO_4 \cdot 7H_2O$ (Showa), 6 mg Na_2MoO_4 (Sigma). Vitamin solution (per liter) includes 100 mg vitamin B_1 (Sigma), 0.5 mg vitamin B_{12} (Sigma) and 0.5 mg biotin (Sigma).

The microalgae were selected for the studies of CO_2 challenge and the high biomass concentration which were cultured in modified f/2 medium in artificial sea water at 26 ± 1 ℃ with an illumination of 300 μmol/m^2/s by white fluorescent light. Modified f/2 medium has following composition (per liter): including 225 mg $NaNO_3$ (Showa), 15 mg $NaH_2PO_4 \cdot H_2O$ (Sigma, Saint Louis, MO, USA), 3 mL of trace metal solution, and 1 mL of vitamin solution

2.3 Experimental system of indoor photobioreactor

The microalgae cells were cultured in photobioreactors with a working volume of 1 L [Chiu et al., 2008]. The photobioreactors were placed in an incubator at 28 ± 1 ℃ with a surface light intensity of approximately 300 μmol/m^2/s provided by continuous, cool-white, fluorescent lights. The photobioreactor was a cylindrical glass column which the diameter and length of the photobioreactor was 5 cm and 80 cm respectively. The gas was supplied from the bottom of the photobioreactor. The flue gas was collected from coke oven in China Steel Corporation, and was exhausted into tank which offered the space to mix it with air. Different flue gas and air volume mixed in the tank would form different ratio gases which would be introduced into bioreactors and utilized by microalgae as nutrient. The gas flow rate was adjusted by using a gas flow meter (Dwyer Instruments, Michigan, IN, USA). The evaluation of tolerance to the flue gas in microalgae cultures, and the microalgae cells in each treatment were sampled every 24 hr for determination of the biomass concentration. The figure about experimental system is presented in **Figure 2-1**.

2.4 Preparation of the inoculums

A stock culture of *Chlorella* sp. (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 ℃ and 300 μmol/m^2/s. After Six days culture, the microalgae 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.

2.5 Experiment design

RSM based on central composite design (CCD) was applied to optimize the experimental conditions for the microalgae cultivation, lipid content, and the compositions of FAME content. Three critical independent parameters affecting microalgae cultivation, lipid content, and the compositions of FAME content: flue gas ratio(x_1), aeration rate(x_2), and initial density(x_3) were selected as the independent variables based on the experiments.

Experimental range and levels of independent variables for microalgae biomass productivity, lipid content, and the compositions of FAME content were presented in **Table 3-7** and **Table 3-8**.

In the optimization process, the responses can be simply related to chosen variables by linear or quadratic models. A quadratic model, which also includes the linear model, is given below:

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_1 x_2 + \beta_5 x_1 x_3 + \beta_6 x_2 x_3 + \beta_7 {x_1}^2 + \beta_8 {x_2}^2 + \beta_9 {x_3}^2 + \varepsilon$$

Where Y is the response and x_1, x_2, and x_3 are the independent variables effects. x_1, x_2, and x_3 are the square effects. x_1x_2, x_1x_3, and x_2x_3 are the interaction effects. β_1, β_2, andβ_3 are the linear coefficients. β_4, β_5, andβ_6 are the interaction coefficients. β_7, β_8, andβ_9 are the squared coefficients. β_0 and ε are the constant and the random error respectively. This model is preferred because a relatively few experimental combinations of the independent variables are adequate to estimate potentially complex response function.

The calculations were based on a least squares analysis. Using this technique, two assumptions are made [Spolaore, 2006b]:

- The expected value of the random error is zero, and its variance is constant over the range of the experimental factors that are used to collect data.
- There is no association of the random error for any one data point with the random error for any other data points.

In the experiment design total twenty experiments were performed in randomized order as required in many design procedures. The design consist of fractional factorial design including the three variables at two levels each augmented by six star points, which α is the distance of the star point from the center, and six center points to evaluate the pure error. Experiment layout and result were analyzed using Design Expert 8.0.6 software and a regression model was proposed. Analysis of Variance (ANOVA) was based on the proposed.

2.6 Lipid extraction

Lipid extraction was according to the modified method previously reported [Chiu et al., 2009b]. The microalgae cells were centrifuged and washed with deionized water twice, and obtained the dry biomass by lyophilization. The dried sample (200 mg) was mixed with methanol/chloroform solution (2/1, v/v) and sonicated for 1 hr. The mixture with methanol/chloroform solution was precipitated and added chloroform and 1 % NaCl solution to give a ratio of methanol, chloroform, and water of 2:2:1. The mixture was centrifuged and the chloroform phase was recovered. Finally, the lipids were weighted after chloroform was removed under vacuum by a rotary evaporator.

2.7 Transesterification

The extracted oil samples were placed in a glass test tube and mixed with 4.0 mL chloroform, 3.4 mL methanol, and 0.6 mL sulfuric acid. The samples were sonicated for 60 minutes. After the reaction was completed, the tubes were removed from the water bath and allowed to cool to room temperature. Then, 2 mL distilled water was added to the tube and thoroughly mixed using a vortex. The samples were allowed to separate, forming a biphasic solution. The organic layer containing FAME was collected and transferred to a pre-weighed glass vial. The solvent was then evaporated using N_2 and heated at 70 ℃ for 40 min. Finally the mass of FAME was determined via weighing.

2.8 Fatty acid profile analysis

The fatty acid composition was determined FOCUS Gas Chromatograph (Thermo Fisher Scientific, Waltham, MA, USA) equipped with an flame ionization detector (FID) and trace GC capillary column (Thermo Fisher Scientific, Waltham, MA, USA), which was a cyanopropylphenyl based phase specifically designed for the separation of FAMEs. A 30 m long column was used with a diameter of 0.32 mm and a 0.25 μm thick film. The amount of sample injected was 2 μL. The stripping gas was nitrogen at a flow rate of 1.3 mL/min, and the injector and detector temperatures were 250 and 280 ℃ respectively. The initial column temperature was 150 ℃ where it remained for 1 min, then rising from 150 to 180 ℃ at

10 ℃/min, remaining at 180 ℃ for 3 min, then rising from 180 to 220 ℃ at 1.5 ℃/min, remaining at 220 ℃ for 1 min, and finally rising from 220 to 260 ℃ at 30 ℃/min, remaining at 260 ℃ for 5 min. The fatty acids were identified by comparison of the retention times with those of the standards using the software Chrom-Card Data System (Thermo Fisher Scientific, Waltham, MA, USA). The analysis of GC profile about the FAME transesterificated from the lipid in microalgae is shown in **Figure 2-2**.

2.9 Analyses

2.9.1 Microalgae cell counting

A direct microscopic count was performed on the sample of microalgae suspension using a Brightline Hemacytometer (BOECO, Hamburg, Germany) and a Nikon Eclipse TS100 inverted metallurgical microscope (Nikon Corporation, Tokyo, Japan).

2.9.2 Measurement of growth rate

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

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

The value y is biomass concentration (g/L). This value was determined according the method previously reported [Chiu et al., 2009a]. Microalgae cells were collected, centrifuged and washed with *deionized* water. The washed microalgae pellet was dried at 105 ℃ for 16 hr; afterward, the dried cells were for dry weight measurement. The value x_1 is optical density measured by the absorbance at 682 nm (A_{682}) in an Ultrospec 3300 pro UV/Visible spectrophotometer (Amersham Biosciences, Cambridge, UK). Each sample was diluted to give an absorbance in the range of 0.1–1.0 if optical density was greater than 1.0.

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

$$\text{Biomass productivity} = \frac{W_f - W_i}{\Delta T}$$

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

2.9.3 Measurements of pH

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.

2.9.4 Measurement of light

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

2.9.5 Determinations of $CO_{2(g)}$

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).

III. Results and Discussion

In order to study the effect of flue gas in microalgae cultivation which *Chlorella* sp. TT-1 was cultivated and find the optimum condition which utilizes flue gas to cultivate microalgae, we constructed an indoor microalgae cultivation system consisting of twenty 1 L bioreactors. We used it to study and discuss the effects of experimental factors in microalgae cultivation with flue gas while the light intensity and cultivated temperature were under control. Furthermore, instead of enormous experiments to test, we cultivated microalgae *Chlorella* sp. TT-1 combining response surface methodology which can reduce the experiment times to diminish the cost, and simulate the experiment formula to get the optimum condition for microalgae cultivation. In my experiment, initial density, aeration rate, flue gas ratio and irradiation time these factors were discussed to study the effect to biomass productivity, lipid content and lipid composition. At last, the optimum microalgae cultivation condition we found was utilized to cultivate microalgae actually even under different irradiation quantity, and it could investigate whether the enhancement of the illumination is necessary in microalgae culture aerated with flue gas under the optimum microalgae cultivation condition and the effect of different illumination density for microalgae cultivation.

3.1 Profile of *Chlorella* sp. TT-1 cultivated with different flue gas ratio

3.1.1 Growth profile in *Chlorella* sp. TT-1 cultures with different flue gas ratios

In this experiment, an isolated thermal- and CO_2-tolerant mutant strain of *Chlorella* sp. TT-1 was used in microalgae cultivation with flue gas aeration. Firstly, flue gas was exhausted into tank and mixed with air. Different flue gas and air volume mixed in the tank would form different ratio gases which would be pumped into bioreactors and utilized by microalgae as nutrient. In order to investigate the appropriate flue gas density for microalgae cultivation, there were 0, 12.5, 25, 50 and 100 % flue gas ratio gases utilized to study the effect of flue gas ratio in microalgae cultivation. *Chlorella* sp. TT-1 in batch culture was incubated for 7 days at 28 ± 2 ℃ and 300 μmol/m^2/s and aerated with different flue gas ratios of mixed gases at 0.2 vvm. The initial inoculums was approximate 0.2 g/L and aerated with 12.5, 25, 50, and 100 % flue gas ratio gases. The air, which indicates the flue gas ratio of 0 %, aerated in microalgae culture was the control group to compare and research the difference of growth profile

between the microalgae cultivation with air and flue gas, which involving rich CO_2 and toxic compounds like nitrogen oxide and sulfur oxide. Cultures were sampled when a stationary phase of growth was reached or a microalgae growth was significantly inhibited. Biomass productivity was calculated from the logarithmic growth phase over 1~3 days batch culture in each experiment.

Figure 3-1 shows growth profile of *Chlorella* sp. TT-1 aerated with different ratios of flue gas. After the 7 days, the cells grew up to stationary phase and the biomass of *Chlorella* sp. TT-1 in 0, 12.5, 25, 50, and 100 % flue gas ratio aeration were 0.515, 0.867, 1.284, 1.243, and 0.847 g/L. The microalgae *Chlorella* sp. TT-1 aerated with 25 and 50 % flue gas had more biomass production than the other microalgae cultivations, and their biomass productivities were 0.421 and 0.357 g/L/day respectively, even the growth rate of microalgae cultivated with 50 % mixture flue gas having a little inhibition at first time. The result of experiment illustrated that *Chlorella* sp. TT-1 has the potential to reduce the CO_2 and utilize it efficiently as carbon source, even the flue gas involving some poisonous compound. However, it was also observed that the flue gas within relatively lower CO_2 was more appropriate for microalgae cultivation even CO_2 mitigation. The mixed flue gases which were diluted to 50 % and 25 % were considered as the more suitable flue gas ratio for microalgae cultivation, and the concentration of CO_2 in mixed flue gas were approximately 14 % and 7 % respectively. The maximum cell concentration of *Chlorella* sp. AG10002 isolated by Ryu was found in the culture with 5 % CO_2 and the cell concentration reached to 2.02 g/L after 6 days, but the CO_2 fixation rate increased less dramatically with the increased CO_2 content [Ryu, 2009]. Chang and Yang 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/day [Chang et al., 2003].

The growth of *Chlorella* sp. TT-1 aerated with 0 % and 12.5 % grew little inhibited due to carbon limitation, and their biomass productivitys were 0.094 and 0.190 g/L/day respectively. The microalgae cultivated with 100 % flue gas was inhibited at first time, and had the biomass productivity 0.301 g/L/day after adapting the condition. Comparing other better microalgae cultivation conditions, aerated with 100 % flue gas even had less growth potential due to the biomass production. It was suggested that total flue gas used in microalgae cultivation was not appropriate. The flue gas involving many compounds like carbon dioxide, nitrogen oxide, and sulfur oxide would affect the pH of culture medium, and this appearance made the pH too low to cultivate microalgae well. In addition, while the

compounds were oxidized by the oxygen, these oxides would produce many radicals which could damage to microalgae cell. These results were not good to microalgae cultivation with flue gas. Therefore, the diluted flue gas is the good strategy to cultivate microalgae when the microalgae cannot tolerant the severe culture condition.

3.1.2 Lipid content and production in *Chlorella* sp. TT-1 cultures with different flue gas ratios

To investigate the effect of flue gas ratio for lipid accumulation, there were 0, 12.5, 25, 50, and 100 % flue gas ratio gases utilized to study the effect of flue gas ratio in lipid accumulation. The air (flue gas ratio is 0 %) aerated in microalgae culture was the control group. *Chlorella* sp. TT-1 in batch culture was incubated for 7 days at 28 ± 2 ℃ and 300 μmol/m^2/s and aerated with different flue gas ratios of mixed gases at 0.2 vvm. *Chlorella* sp. TT-1 at stationary phase was collected after 7 days to measure lipid content.

Lipid content and production of *Chlorella* sp. TT-1 cultures in different flue gas ratio gases are illustrated in **Table 3-1**. Lipid content of *Chlorella* sp. TT-1 cultures in 0, 12.5, 25, 50, and 100 % flue gas ratio aeration were 32.18, 41.55, 40.23, 36.53, and 36.93 %. Comparing with the microalgae cultivation aerated with air, the microalgae cultures with flue gas had more lipid content in the microalgae cells, and the variance of lipid content between air and flue gas was approximately 10~15 %. It explains microalgae cultivated with flue gas could enhance the lipid accumulation. In addition, the microalgae cells could accumulate the lipid content above 40 % when *Chlorella* sp. TT-1 cultivated with 12.5 and 25 % flue gas, and their lipid production were 0.360 g/L and 0.517 g/L. Whereas, *Chlorella* sp. TT-1 cultivated with 50 % flue gas had better lipid production 0.454 g/L than microalgae cultivated with 12.5 % flue gas. Increasing lipid accumulation will not result in increased lipid production as biomass productivity and lipid accumulation are not necessarily correlated. Lipid accumulation refers to increased concentration of lipids within the microalgae cells without consideration of the overall biomass production. Lipid production takes into account both the lipid concentration within cells and the biomass produced by these cells and is therefore a more useful indicator of the potential costs of liquid biofuel production [Rodolfi et al., 2008; Sheehan et al., 1998a, b]. In summary, the lipid productivity plays important role to get the most lipid quantity and *Chlorella* sp. TT-1 cultivated in 25 % had the most lipid quantity.

3.1.3 Lipid composition in *Chlorella* sp. TT-1 cultures with different flue gas ratios

Lipid compositions of *Chlorella* sp. TT-1 under different flue gas ratios cultivation were analyzed by Gas chromatography. The length of fatty acid chain plays an important role to decide the characteristics of biodiesel such as pour point, boiling point and so on. C16:0, C18:0, C18:1, C18:2 and C18:3 are usual fatty acid for biodiesel contents [Knothe, 2008]. **Table 3-2** shows the C16:0, C18:0, C18:1, C18:2 and C18:3 of *Chlorella* sp. TT-1 in each experiment condition. However, there was no difference between the FAME content of *Chlorella* sp. TT-1 cultivated with different flue gas ratios. In summary, the FAME content of *Chlorella* sp. TT-1 were approximately 40 %.

Instead of *Chlorella* sp. TT-1, there are other microalgae spices which are able to stimulate the specific fatty acid methyl ester (FAME) for FAME by changing microalgae cultivation condition. Ota found that at CO_2 concentrations between 20 to 50 %, the total fatty acid content of *Chlorococcum littorale* decreased [Ota et al., 2009]. High CO_2 levels (30 to 50 %) have been shown to favor the accumulation of total lipids and polyunsaturated fatty acids in certain microalgae [Chiu et al., 2008; Tang et al., 2011]. Shifting *Chlorella* from limiting to higher CO_2 concentrations increased the proportion of saturated fatty acids [Tsuzuki et al., 1990; Chiu et al., 2011].

3.2 Profile of *Chlorella* sp. TT-1 cultivated at different aeration rate

The effects of carbon oxide uptake are different under different aeration rates. In addition, different aeration rates will make different ratios CO_2 dissolve in medium, and this phenomenon will improve the efficiency to utilize CO_2 as carbon source for microalgae. Therefore, the experimental factor aeration rate was taken in account in my experiment to investigate the effect of different aeration rate with flue gas.

3.2.1 Growth profile in *Chlorella* sp. TT-1 cultures with different aeration rates

To research the effect of microalgae cultivation at different aeration rates, *Chlorella* sp. TT-1 in batch culture was incubated for 7 days at 28 ± 2 ℃ and 300 μmol/m^2/s and aerated with different aeration rate of mixed gases with 25 % flue gas ratio. The initial inoculums (approximate 0.2 g/L) was cultured and aerated at 0.1, 0.2 and 0.3 vvm aeration rates. Cultures were sampled when a stationary phase of growth was reached or a microalgae

growth was significantly inhibited. Biomass productivity was calculated from the logarithmic growth phase over 1~3 days batch culture in each experiment.

Figure 3-2 shows growth profile of *Chlorella* sp. TT-1 aerated with different aeration rates. After the 7 days, the cells grew up to stationary phase and the biomass of *Chlorella* sp. TT-1 in 0.1, 0.2 and 0.3 vvm aeration rate were 0.775, 0.842 and 0.897 g/L. This figure explains different aeration rates would not influence the growth profile of *Chlorella* sp. TT-1 significantly, and the final biomass variance between the most and the least was only 0.122 g/L. The biomass productivities at 0.1, 0.2 and 0.3 vvm were 0.270, 0.279, and 0.286 g/L/day, respectively.

In other researches of aeration rate for microalgae cultivation, Ryu demonstrated that the high aeration rate resulted in the high cell concentration of *Chlorella sp*. AG10002 and the low CO_2 utilization efficiency. The optimum aeration rate in consideration of both the productivity and the CO_2 utilization efficiency was 0.20 vvm [Ryu et al., 2009]. Also, Chiu investigated that significant higher of CO_2 fixation rate was also found when the aeration rate decreases in the result of the increasing of CO_2 absorption from bubbling gas. This was caused by the increase of surface area per unit gas volume of the bubble which would also enhance the CO_2 removal efficiency [Chiu et al., 2009b].

3.2.2 Lipid content and production in *Chlorella* sp. TT-1 cultures with different aeration rates

In order to study the effect of aeration rate for lipid accumulation, the aeration rates at 0.1, 0.2, and 0.3 vvm were aerated in *Chlorella* sp. TT-1. *Chlorella* sp. TT-1 in batch culture was incubated for 7 days at 28 ± 2 ℃ and 300 μmol/m^2/s and cultivated at different aeration rates with the 25 % flue gas ratio. *Chlorella* sp. TT-1 at stationary phase was collected after 7 days to measure lipid content.

Lipid content and production of *Chlorella* sp. TT-1 cultures at different aeration rates are illustrated in **Table 3-3**. From this table, the lipid contents of *Chlorella* sp. TT-1 with 0.1, 0.2 and 0.3 vvm aeration rates were 36.12, 37.61 and 39.90 % respectively, and the lipid production of *Chlorella* sp. TT-1 with 0.1, 0.2 and 0.3 vvm aeration rate were 0.280, 0.317 and 0.358 g/L. The microalgae cultivated with different aeration rates would aerate in the different quantity of carbon dioxide, and the higher aeration rate used in microalgae cultivation would aerate more CO_2 in the bioreactor. On the other hand, the different aeration

rate would lead to different CO_2 fixation efficiency which affects the physiology in microalgae cell and various levels of turbulence to influence not only gas and mass transfer but also shear stress [Kumar et al., 2010]. These experiment factors induced by aeration rate brought about the different lipid accumulation in microalgae cell, and the result of the experiment was obtained the lipid content and production in microalgae cells increased with the increasing aeration rate.

3.2.3 Lipid composition in *Chlorella* sp. TT-1 cultures with different aeration rates

Lipid compositions of *Chlorella* sp. TT-1 under different aeration rates cultivation were analyzed by Gas chromatography, and it is illustrated in **Table 3-4**. It showed the contents of FAME compositions slightly increase with high aeration rate, and the variance is 4~5 %.

For the purpose of FAME production from microalgae biomass, lipid productivity should be a selection parameter and a critical variable for the evaluation of microalgae species and culture operation [Chiu et al., 2009a; Griffiths et al., 2009; Chen et al., 2011]. Our results suggested that a *Chlorella* sp. TT-1 culture aerated with flue gas is a suitable candidate for microalgae lipid production. The most common fatty esters in biodiesel are palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3) [Knothe, 2008]. The fatty acid profile of *Chlorella* sp. TT-1 consists mainly of C_{16} and C_{18} fatty acids, and the major composition of the fatty acids in the microalgae strain was C16:0. C16:0 is the major saturated fatty acid in 12 microalgae strains that represent 8 classes, including *Chlorophyceae* [Patil et al., 2007]. The fatty acids of *Chlorella* sp. TT-1 were mainly saturated. In summary for the microalgae cultivation with flue gas under different flue gas ratio and aeration rate, the effect of flue gas on fatty acid compositions was not clear and would be further investigated.

3.3 Microalgae cultivation under the illumination of full and half illumination aerated with flue gas

3.3.1 Growth profile of *Chlorella* sp. TT-1 under full and half illumination

In order to investigate whether the illumination is necessary in microalgae culture aerated with flue gas, the *Chlorella* sp. TT-1 was cultivated under full and half illumination, which means 24 hr irradiation and 12 hr irradiation (10:00 ~ 22:00). *Chlorella* sp. TT-1 in

batch culture was incubated for 7 days at 28 ± 2 ℃ and 300 μmol/m^2/s and aerated with different flue gas ratios of mixed gases at 0.2 vvm. The initial inoculums (approximate 0.2 g/L) was cultured and aerated with 25 % flue gas ratio gases. The air (flue gas ratio is 0 %) aerated in microalgae culture was the control group. Microalgae cultivation with full illumination was 24 hr irradiation with light, and half illumination was 12 hr irradiation while shielded the light at 22:00 and discovered it at 10:00. The cultures were sampled at the two time described as mentioned above, and ceased sampling when a stationary phase of growth was reached or a microalgae growth was significantly inhibited. Biomass productivity was calculated from the logarithmic growth phase over 1~3 days batch culture in each experiment.

The result of the *Chlorella* sp. TT-1 cultivated with flue gas under full and half illumination are demonstrated in **Figure 3-3**. After 7 days, the cells grew up to stationary phase and the biomass production of *Chlorella* sp. TT-1 in full and half illumination were 1.116 g/L and 0.517 g/L respectively. The biomass productivity of *Chlorella* sp. TT-1 in full and half illumination were 0.299 g/L/day and 0.137 g/L/day respectively. The microalgae cultivation with flue gas under full illumination was obviously better than half illumination, and the variance of biomass production was approximately 0.6 g/L. Otherwise, the biomass of *Chlorella* sp. TT-1 cultivated with flue gas under half illumination even reduced during the microalgae aerated with flue gas was shielded from irradiation. The reason why the biomass decreases is the microalgae cell had no ability to do photosynthesis and other metabolism without illumination, and it would not metabolize the materials in flue gas. Nagase showed the result that green algae assimilate nitrogen via a metabolic pathway which is dependent on energy supply from photosynthesis; thus, the uptake of nitrogen source into the cells was also dependent on photosynthesis [Nagase et al., 2001]. Whereas, some materials in flue gas were damage to microalgae cell, even making the pH of culture medium more acidic, and it was not the appropriate condition for microalgae cultivation. In summary, the better irradiation time was full illumination for microalgae cultivation, and the light should be devised when the microalgae cultivated with flue gas. In the future, the outdoor microalgae cultivation which only has half illumination should set up the additional light device for illumination or switch off the flue gas to prevent flue gas aerating in bioreactor to damage the microalgae cell.

3.3.2 Lipid content and production of *Chlorella* sp. TT-1 under full and half illumination

The *Chlorella* sp. TT-1 was cultivated under full and half illumination to study whether the illumination is critical for lipid accumulation of microalgae cell when microalgae aerated

with flue gas. The microalgae *Chlorella* sp. TT-1 in batch culture was incubated for 7 days at 28 ± 2 ℃ and 300 μmol/m^2/s and aerated with different flue gas ratios of mixed gases at 0.2 vvm. The initial inoculums (approximate 0.2 g/L) was cultured and aerated with 25 % flue gas ratio gases. The air (flue gas ratio is 0 %) aerated in microalgae culture was the control group. The full illumination and half illumination mean 24 hr irradiation and 12hr irradiation (10:00 ~ 22:00). *Chlorella* sp. TT-1 at stationary phase was collected after 7 days to measure lipid content.

The experimental result is shown in **Table 3-5**. From the table, the lipid content of *Chlorella* sp. TT-1 under full and half illumination were 36.90 % and 14.53 %, and the lipid production were 0.412 g/L and 0.083 g/L respectively. Obviously, the lipid in microalgae cell under full illumination was all more than it cultivated under half illumination, and the variance between two illumination modes was approximately 20 % while the variance of lipid productivity was above 0.3 g/L. In addition, we could observe the phenomenon also expressed in the lipid content of microalgae which cultivated with air. It is proven that the illumination for microalgae cultivation plays the very important role on both biomass production and lipid accumulation. Microalgae operate photosynthesis using the illumination as the energy to fix carbon dioxide which produces the ingredient for lipid synthesis.

3.3.3 Lipid composition of *Chlorella* sp. TT-1 under full and half illumination

The *Chlorella* sp. TT-1 was cultivated under full and half illumination to study how the illumination affected the lipid composition of microalgae cell when microalgae aerated with flue gas. The microalgae *Chlorella* sp. TT-1 in batch culture was incubated for 7 days at 28 ± 2 ℃ and 300 μmol/m^2/s and aerated with different flue gas ratios of mixed gases at 0.2 vvm with 25 % flue gas ratio gases. The air (flue gas ratio is 0 %) aerated in microalgae culture was the control group. The full illumination and half illumination mean 24 hr irradiation and 12 hr irradiation (10:00 ~ 22:00), and lipid compositions of *Chlorella* sp. TT-1 under full and half illumination were analyzed by Gas chromatography.

The experiment result is displayed in **Table 3-6**. *Chlorella* sp. TT-1 consists mainly of C_{16} and C_{18} fatty acids, and the major composition of the fatty acids in the microalgae strain was C16:0. C16:0 is the major saturated fatty acid in 12 microalgae strains that represent 8 classes, including *Chlorophyceae* [Patil et al., 2007]. The FAME ratio of *Chlorella* sp.TT-1 aerated with flue gas under full illumination was similar to it cultivated under half

illumination, but the FAME content in microalgae cell under full illumination microalgae cultivation was more because of the much more lipid content in it. On the other hand, the saturated fatty acid (C16:0) accounted for the most part in *Chlorella* sp. TT-1 cultivated under half illumination, while the microalgae had more long and unsaturated fatty acid (C18:1, C18:2, and C18:3) under full illumination. The properties of a biodiesel fuel, including its ignition quality, combustion heat, cold filter plugging point (CFPP), oxidative stability, viscosity, and lubricity, are determined by the structure of its component fatty esters. High levels of saturated fatty acids tend to increase the stability of biodiesel because unsaturated fatty acids result in poor oxidative stability. The effect of flue gas on fatty acid compositions was not clear and would be further investigated.

3.4 The optimization of *Chlorella* sp. TT-1 aerated with flue gas in biomass productivity, lipid productivity, and FAME productivity

In this study, three experimental factors were studied to evaluate the approximate polynomial for all dependent variables, explaining their effects on biomass productivity, lipid productivity, and FAME productivity. RSM and CCD were then used to optimize microalgae cultivation. Optimization design process involves mainly four major steps [Zhang, 2009] :

(1) Perform statistically designed experiments according to the experimental plan.

(2) Propose the mathematical model based on the experimental results and elaborate the result of analysis of variance (ANOVA).

(3) Check the adequacy of the model through diagnostic plots.

(4) Predict the response and confirm the model.

The levels of the variables for the CCD experiments were selected according to the results of the previous experiments. The center point of the corresponding experiment factors were selected to be initial density 0.3 g/L, aeration rate 0.2 vvm, and flue gas ratio 50 %, and both high and low levels are narrated in **Table 3-7**. The experimental design layout produced from the software Design-expert 8.0.6.with twenty experiment runs was presented in **Table 3-8**.

3.4.1 The optimization of *Chlorella* sp. TT-1 in biomass productivity

In order to study the interaction between the three experiment factor and the optimum condition for microalgae cultivation, *Chlorella* sp. TT-1 in batch culture was incubated for 7 days at 28 ± 2 ℃ and 300 μmol/m^2/s. The experiment runs are demonstrated as **Table 3-8**. Biomass productivity was calculated from the logarithmic growth phase over 1~3 days batch culture in each experiment. Data obtained from the experiments (**Table 3-8**) were analyzed by using software Design-expert 8.0.6. The corresponding second-order response model for Eq. (1) that was founded after analysis for the regression was:

$$\text{Biomass productivity} = -0.40917 + 3.20357 \times A - 3.42913 \times A^2 - 0.45958 \times C^2 \quad (1)$$

Adequacy check of the proposed model is an important part of the analysis procedure. Good adequacy can ensure that the approximating model provides an adequate approximation to the real system, or it may give poor or misleading results [Körbahti et al., 2008]. The diagnostic plots shown in **Figure 3-4 and 3-5** were used to estimate the adequacy of regression model. The actual biomass productivity and predicted value plot for the microalgae of *Chlorella* sp. TT-1 is shown in **Figure 3-4**. The actual biomass productivity was the measured value for a particular run and the predicted value was evaluated from the model. In designed experiments, R^2 is a measure of the amount of reduction in the variability of the response obtained by using the independent variables in the model. A large value of R^2 does not imply that the regression model is a good one. However, adj. R^2 is preferred to be used to determine the fit of a regression model as it does not always increase when variables are added. As shown in **Figure 3-4**, a good agreement has been obtained between the predicted biomass productivity and the actually experimental value with R^2 and adj. R^2 of 0.9400 and 0.8860, respectively, which indicated that the proposed model had adequate approximation to the actual value and ensured a satisfactory adjustment of the proposed model with more than 88.6 %. Residuals indicate how well the model satisfies the assumptions of ANOVA, whereas the internally studentized residuals measure the standard deviations separating the actual and predicted values [Liu et al., 2005]. The internally studentized residuals and normal % probability plot for the biomass productivity of *Chlorella* sp. TT-1 is shown in **Figure 3-5**. The data points lying on the straight line shows that neither response transformation was required nor there was any apparent problem with normality. This indicated that Eq. (1) is a suitable model to describe the response of the experiment.

To determine the optimal condition of biomass productivity and the relationship between the response and the significant variables, statistical analyses of variance (ANOVA) is performed through a joint test of three parameters (**Table 3-9**). ANOVA for biomass productivity showed that fitted quadratic response surface model was highly significant with F-test = 17.4050 ($p < 0.001$) as shown in **Table 3-9**. In addition, the lack-of -fit test is also a viewpoint to see whether the simulated regression fitted the actual experiment, and the F-test of 3.1812 ($p = 0.1149$) showed it was not significant. That the result of lack-of-fit was not significant is good for the regression model. We do not want the model is lack-of-fit.

A *p*-value is the indicator of the significance of the test, whose value below 0.05 indicates that test parameter is significant at 5% level of significance. The *p*-value is used for the evaluation of model significance. A very significant model has a *p*-value below 0.001, a significant model below 0.01 and an almost significant model below 0.05. From **Table 3-10**, the experiment factor of initial density (A) had a strongly linear effect on the response. In contrast, when the A was kept at the center of the levels, the aeration rate (B) and flue gas ratio (C), especially aeration rate (*p*-value = 0.9737) had a negligible linear effect on the response. From the regression coefficients and p-value, the quadratic term of initial density (A) and flue gas ratio (C) had highly significant effects on the biomass productivity ($p < 0.05$). The optimal conditions of the three factors for microalgae cultivation were predicted using the optimization function of the Design-Expert version 8.0.6 software.

The 3D response surface plot was generally the graphical representation of the regression equation. **Figure 3-6** represents the 3D response surface plots for the optimization of the biomass productivity of *Chlorella* sp. TT-1 for microalgae cultivation. Each figures presented the effect of two variables on the biomass productivity, while other two variables were held at zero level.

Figure 3-6A shows the response surface plot as function of initial density, aeration rate, and interaction on the biomass productivity. The biomass productivity increased with the increase of the initial inoculums, and the slightly increase of aeration rate. The more initial cultivation density applied the ability to endure high aeration rate and high flue gas ratio, and it would support the microalgae more efficient to utilize the carbon dioxide in flue gas. It has been investigated in many researches. For example, the cell concentration of *Chlorella sp*. AG10002 and *Chlorella* sp. TT-1 were improved with the increased CO_2 concentration. However, relatively lower CO_2 fixation rate was observed than expected at the high CO_2

concentration of 5.0 % and the high CO_2 concentration was to be not effective in view of the CO_2 fixation in the microalgae cultivation of *Chlorella sp*. AG10002 [Chiu et al., 2011; Ryu et al., 2009]. However, the self shading may be happened in high initial culture density, and it is mention in literature review. The effect of varying initial density and flue gas ratio are shown in **Figure 3-6B**. It was suggested that the increasing flue gas ratio utilized by *Chlorella* sp. TT-1 was proper with increasing initial cultivation density, but the flue gas ratio could not exceed more than 55 % which is also demonstrated in **Figure 3-6C**. The experiment result fits the outcome which shows in the past sections about the effects of different flue gas ratio in microalgae cultivation. As the result, the optimized biomass productivity of *Chlorella* sp. TT-1 is 0.486 g/L/day when the initial density of 0.37 g/L aerated with 75 % flue gas ratio at 0.30 vvm aeration rate. The results of experiments showed that this model founded by the software was successfully built and had good validity.

3.4.2 The optimization of *Chlorella* sp. TT-1 in lipid content

In order to study the interaction between the three experiment factor and the optimum condition for lipid content, *Chlorella* sp. TT-1 in batch culture was incubated for 7 days at 28 ± 2 ℃ and 300 μmol/m^2/s. The experiment runs are demonstrated as **Table 3-8**. *Chlorella* sp. TT-1 at stationary phase was collected after 7 days to measure lipid content. The Data of lipid content in *Chlorella* sp. TT-1 obtained from the experiments (**Table 3-8**) were analyzed by using software Design-expert 8.0.6. The corresponding second-order response model for Eq. (2) that was founded after analysis for the regression was:

$$\text{Lipid content} = -0.70869 + 209.78387 \times A - 281.74265 \times A^2 - 177.88269 \times B^2 + 53.84600 \times C^2 \quad (2)$$

The diagnostic plots shown in **Figure 3-7 and 3-8** were used to estimate the adequacy of regression model. The actual lipid content and predicted value plot for the microalgae of *Chlorella* sp. TT-1 is shown in **Figure 3-7**. The actual lipid content was the measured value for a particular run and the predicted value was evaluated from the model. As shown in **Figure 3-7**, a good agreement has been obtained between the predicted lipid content and the actually experimental value with R^2 and adj. R^2 of 0.8705 and 0.7539, respectively, which indicated that the proposed model had adequate approximation to the actual value and ensured a satisfactory adjustment of the proposed model with more than 75.4 %. The internally studentized residuals and normal % probability plot for the lipid content of *Chlorella* sp. TT-1

is shown in **Figure 3-8**. The data points lying on the straight line showed that neither response transformation was required nor there was any apparent problem with normality. This indicated that Eq. (2) was a suitable model to describe the response of the experiment.

To determine the optimal condition of lipid content and the relationship between the response and the significant variables, statistical analyses of variance (ANOVA) is performed through a joint test of three parameters (**Table 3-11**). ANOVA for biomass productivity shows that fitted quadratic response surface model was highly significant with F-test = 7.47 (p = 0.0021) as shown in **Table 3-11**.

A p-value is the indicator of the significance of the test, whose value below 0.05 indicates that test parameter is significant at 5 % level of significance. The p-value is used for the evaluation of model significance. A very significant model has a p-value below 0.001, a significant model below 0.01 and an almost significant model below 0.05. From **Table 3-12**, the experiment factor of initial density (A) had a strongly linear effect on the response. In contrast, when the A was kept at the center of the levels, the aeration rate (B) and flue gas ratio (C) had a negligible linear effect on the response, and the p-values were 0.8478 and 0.7144 respectively. From the regression coefficients and p-value, the quadratic term of initial density (A), aeration rate (B), and flue gas ratio (C) all had highly significant effects on the lipid content ($p < 0.05$). The optimal conditions of the three factors for lipid content were predicted using the optimization function of the Design-Expert version 8.0.6 software.

The 3D response surface plot about the lipid content of *Chlorella* sp. TT-1 is demonstrated in **Figure 3-9. Figure 3-9A** shows the response surface plot as function of initial density, aeration rate, and interaction on the biomass productivity. The lipid content slightly increased with the increase of the aeration rate, which is also demonstrated in **Figure 3-9C**, and the suggested initial density could not more than 0.36 g/L. The result also is mentioned before that the increasing aeration would enhance lipid accumulation in microalgae cell. The effect of varying initial density and flue gas ratio is shown in **Figure 3-9B**. It is also suggested that the inoculums of microalgae quantity could not be more than 0.36 g/L, and lipid content increased with the increase of flue gas ratio after 55 % due to the interaction between initial density and flue gas ratio. As the result, the optimized lipid production of *Chlorella* sp. TT-1 is 0.216 g/L (44 % lipid of biomass) when the initial density of 0.35 g/L aerated with 75 % flue gas ratio at 0.24 vvm aeration rate. The results of experiments showed that this model founded by the software was successfully built and had good validity.

3.4.3 The optimization of *Chlorella* sp. TT-1 in FAME content

In order to study the interaction between the three experiment factor and the optimum condition for microalgae cultivation. The *Chlorella* sp. TT-1 in batch culture was incubated for 7 days at 28 ± 2℃ and 300 μmol/m^2/s. The experiment runs are demonstrated as **Table 3-8**. Lipid compositions of *Chlorella* sp. TT-1 were analyzed by Gas chromatography in each experiment. The data of FAME composition in *Chlorella* sp. TT-1 obtained from the experiments (**Table 3-8**) were analyzed by linear multiple regression using software Design-expert 8.0.6. The corresponding second-order response model for Eq. (3) that was founded after analysis for the regression was:

$$\text{FAME content} = -16.26776 + 180.21538 \times A - 305.43241 \times A^2 - 257.14431 \times B^2 \quad (3)$$

The diagnostic plots shown in **Figure 3-10 and 3-11** are used to estimate the adequacy of regression model. The actual FAME content and predicted value plot for the microalgae of *Chlorella* sp. TT-1 is shown in **Figure 3-10**. The actual FAME content was the measured value for a particular run and the predicted value was evaluated from the model. As shown in **Figure 3-10**, a good agreement has been obtained between the predicted biomass productivity and the actually experimental value with R^2 and adj. R^2 of 0.8564 and 0.7271, respectively, which indicated that the proposed model had adequate approximation to the actual value and ensured a satisfactory adjustment of the proposed model with more than 72.7 %. The internally studentized residuals and normal % probability plot for the FAME content of *Chlorella* sp. TT-1 is shown in **Figure 3-11**. The data points lying on the straight line showed that neither response transformation was required nor there was any apparent problem with normality. This indicated that Eq. (3) was a suitable model to describe the response of the experiment.

To determine the optimal condition of FAME content and the relationship between the response and the significant variables, statistical analyses of variance (ANOVA) is performed through a joint test of three parameters (**Table 3-13**). ANOVA for FAME content shows that fitted quadratic response surface model was highly significant with F-test = 6.62 ($p < 0.001$) as shown in **Table 3-13**. In addition, the lack-of -fit test is also a viewpoint to see whether the simulated regression fitted the actual experiment, and the F-test of 1.5401 ($p = 0.3236$) showed it was not significant. That the result of lack-of-fit was not significant is good for the regression model. We do not want the model is lack-of-fit.

A p-value is the indicator of the significance of the test, whose value below 0.05 indicates that test parameter is significant at 5 % level of significance. The *p*-value is used for the evaluation of model significance. A very significant model has a *p*-value below 0.001, a significant model below 0.01 and an almost significant model below 0.05. From **Table 3-14**, the experiment factor of initial density (A) had a strongly linear effect on the response. In contrast, when the A was kept at the center of the levels, the aeration rate (B) and flue gas ratio (C) had a negligible linear effect on the response. From the regression coefficients and *p*-value, the quadratic term of initial density (A) and aeration rate (B) had highly significant effects on the FAME content ($p < 0.05$). The optimal conditions of the three factors for FAME content were predicted using the optimization function of the Design-Expert version 8.0.6 software.

The 3D response surface plot about the FAME content of *Chlorella* sp. TT-1 is demonstrated in **Figure 3-12**. **Figure 3-12A** shows the response surface plot as function of initial density, aeration rate, and interaction on the biomass productivity. It suggested that the inoculums of microalgae quantity could not be more than 0.34 g/L and less than 0.30 g/L, which was also showed in **Figure 3-12B**, and it could produce more FAME content in microalgae cell. The effects of varying aeration rate and flue gas ratio are shown in **Figure 3-12C**. The FAME content slightly increased with the increase of the aeration rate and the flue gas ratio. As the result, the optimized FAME production of *Chlorella* sp. TT-1 is 0.157 g/L (32 % FAME of biomass) when the initial density of 0.37 g/L aerated with 74 % flue gas ratio at 0.25 vvm aeration rate. The results of experiments showed that this model founded by the software was successfully built and had good validity.

3.4.4 The predicted value and experimental productivity under optimum microalgae cultivation condition

In this section, each optimum condition for biomass productivity, lipid productivity and FAME productivity was operated to confirm whether the experimental results are workable or not, and the result is shown in **Figure 3-15**. The experimental biomass productivity with three repeats were 0.465, 0.427 and 0.387 g/L/day comparing to the predicted value which was 0.486 g/L/day; the experimental lipid production with three repeats were 0.195, 0.178, and 0.151 g/L/day respectively comparing to the predicted value which was 0.216 g/L/day; the experimental FAME production with three repeats were 0.163, 0.145, and 0.141 g/L/day respectively comparing to the predicted value which was 0.157 g/L/day.

3.5 Different light illumination used in the optimum microalgae cultivation

3.5.1 Growth profile of *Chlorella* sp. TT-1 cultivated with different illuminations

In order to investigate whether the enhancement of the illumination is necessary in microalgae culture aerated with flue gas under the optimum microalgae cultivation condition and the effect of different illumination density for microalgae cultivation, the isolated thermal- and CO_2-tolerant mutant strain of *Chlorella* sp. TT-1 was used in microalgae cultivation with flue gas aeration. *Chlorella* sp. TT-1 was cultivated under the illumination of 300, 500, and 700 μmol/m^2/s, while the 300 μmol/m^2/s was the control group to compare the experiment groups with enhanced irradiation which were 500 and 700 μmol/m^2/s. *Chlorella* sp. TT-1 in batch culture was incubated for 7 days at 28 ± 2 ℃. The microalgae was cultivated under the optimum microalgae cultivation condition which the initial density of 0.37 g/L aerated with 75 % flue gas ratio at 0.30 vvm aeration rate. The cultures were sampled when a stationary phase of growth was reached or a microalgae growth was significantly inhibited. Biomass productivity was calculated from the logarithmic growth phase over 1~3 days batch culture in each experiment.

Figure 3-13 shows growth profile of *Chlorella* sp. TT-1 cultivated with different illuminations. After the 7 days, the cells grew up to stationary phase and the biomass of *Chlorella* sp. TT-1 with the illuminations of 300, 500, and 700 μmol/m^2/s were 0.846, 1.015, and 0.451 g/L, respectively. The microalgae cultivation cultivated with 500 μmol/m^2/s produced the most microalgae biomass which the biomass productivity was 0.390 g/L/day comparing the microalgae cultivation irradiated with 300 μmol/m^2/s which the biomass productivity was 0.335 g/L/day. The phenomenon of photoinhibition was happened when the microalgae cultivated with 700 μmol/m^2/s, and the lower biomass productivity was 0.120 g/L/day comparing with the microalgae cultivated with 300 μmol/m^2/s.

Simionato investigated the effect of different illuminations for microalgae cultivation by *Nannochloropsis gaditana.* It showed cultures exposed to very low light showed slower growth, clearly imputable to a limitation in the energy available to support metabolism. On the other extreme, cultures exposed to very strong light reached the stationary phase earlier and final cells number was lower [Simionato, 2011]. Above the saturation of light intensity, a further increase in light level actually reduces the biomass growth rate. This phenomenon is known as photoinhibition. For example, the light saturation constants for microalgae

Phaeodactylum tricornutum and *Porphyridium cruentum* were 185 μE/m^2/s and 200 μE/m^2/s [Chisti, 2007], respectively. Microalgae become photoinhibited at light intensities only slightly greater than the light level at which the specific growth rate peaks. Photoinhibition results from generally reversible damage to the photosynthetic apparatus, as a consequence of excessive light [Chisti, 2007].

3.5.2 Lipid content and production of *Chlorella* sp. TT-1 cultivated with different illuminations

The condition of microalgae cultivation in this part is not specific and optimum for lipid content and production, so the experimental purpose to this experiment is to study the effect of different illuminations for lipid accumulation when microalgae aerated with flue gas. The microalgae *Chlorella* sp. TT-1 was cultivated under the microalgae cultivation condition which the initial density of 0.37 g/L aerated with 75 % flue gas ratio at 0.30 vvm aeration rate. The microalgae in batch culture was incubated for 7 days at 28 ± 2 ℃ and the illuminations of 300, 500, and 700 μmol/m^2/s were irradiated in the experiment. *Chlorella* sp. TT-1 at stationary phase was collected after 7 days to measure lipid content.

The experiment result is demonstrated in the **Table 3-16**. The lipid content of *Chlorella* sp. TT-1 under the optimum microalgae condition were 39.10, 48.68, and 42.25 % respectively, and the illumination of 500 μmol/m^2/s which also stimulated the most biomass production could enhance the lipid accumulation from the **Table 3-16**. It could attain the variance of 4.5 ~ 9.5 % roughly, and the lipid production was 0.494 g/L. There was the phenomenon of photoinhibition when the microalgae cultivated with the illumination of 700 μmol/m^2/s which the lipid production was 0.195 g/L compared with the microalgae cultivated with the illumination of 300 μmol/m^2/s which the lipid production was 0.331 g/L.

The same experiment result was also illustrated in *Nannochloropsis* sp.. Rodolfi L et al. displayed the increase of illumination could raise the lipid content in *Nannochloropsis* sp. cell. When the irradiance increased from 115 to 230 μmol/m^2/s, it would lead the lipid productivity from 0.61 to 0.85 g/L/day and FA from 14.7 % to 19.6 % [Rodolfi et al., 2008]. In summary, illumination plays the important role in lipid accumulation.

3.5.3 Lipid composition and production of *Chlorella* sp. TT-1 cultivated with different illuminations

The condition of microalgae cultivation in this part is not specific and optimum for

FAME content, so the experimental purpose to this experiment is to study the effect of different illuminations for FAME composition aerated with flue gas. T The microalgae *Chlorella* sp. TT-1 was cultivated under the microalgae cultivation condition which the initial density of 0.37 g/L aerated with 75% flue gas ratio at 0.30 vvm aeration rate. The microalgae in batch culture was incubated for 7 days at 28 ± 2 ℃ and the illuminations of 300, 500, and 700 μmol/m^2/s were irradiated in the experiment. Lipid compositions of *Chlorella* sp. TT-1 were analyzed by Gas chromatography.

The experiment result is demonstrated in the **Table 3-17**. From this table, the C16:0 content of *Chlorella* sp. TT-1 under the optimum microalgae cultivation condition were 38.64, 46.73, and 42.25 % respectively, and the C18:1 and C18:2 content in *Chlorella* sp. TT-1 were 15.68, 13.02, and 12.28 % and 18.55, 14.22, and 17.09 %, respectively. The illumination of 500 μmol/m^2/s which also stimulated the most biomass production and lipid content had more C16:0 content, and it mean higher illumination would induce microalgae to synthesize the carbon compound of shorter chain like C16:0 comparing with the FAME composition of microalgae which was cultivated with the illumination of 300 μmol/m^2/s. Solovchenko investigated the effect of illumination on the microalgae *Parietochloris incise*, and it found the triacylglycerol and even polyunsaturated fatty acid would be accumulated with the high illumination of microalgae cultivation [Solovchenko et al., 2008].

IV. Conclusions

In the recent years, global warming becomes more serious problem due to the increasing carbon dioxide (CO_2) accumulated in the atmosphere, The plant steel for industry plays the important role in releasing flue gas which is CO_2-rich. Microalgae is the candidate to solve the problem by photosynthesis, which use sun light as energy source to convert water and CO_2 into biomass, and it can consume CO_2 to reduce the CO_2 emission. We utilized these mutant microalgae to reduce the CO_2 in flue gas aerated from coke oven of a steel plant and produce microalgae biomass which can extract oil to produce FAME. Furthermore, we cultivate microalgae combined with response surface methodology to get the optimized cultivation condition under the specific initial density, aeration rate, and flue gas ratio.

To investigate the effect of flue gas ratio, there were 0, 12.5, 25, 50, and 100 % flue gas ratio gases utilized to study the effect of flue gas ratio in microalgae cultivation. The microalgae *Chlorella* sp. TT-1 aerated with 25 % flue gas had more biomass production, which is 1.284 g/L, than the other microalgae cultivations, and its biomass productivity were 0.421 g/L/day. Lipid content of *Chlorella* sp. TT-1 cultures with 12.5, 25 % flue gas ratio aeration were 41.55 % and 40.23 %. By all accounts, *Chlorella* sp. TT-1 cultivated in 25 % had the most lipid quantity due to biomass production and lipid content. In the part of FAME, there was no difference between the FAME content of *Chlorella* sp. TT-1 cultivated with different flue gas ratios, but lipid enhancement will increase the FAME content in microalgae cell simultaneously.

The aeration rate of 0.1, 0.2, and 0.3 vvm were aerated in microalgae culture to study the effect of aeration rate with flue gas. The biomass production of *Chlorella* sp. TT-1 at 0.1, 0.2, and 0.3 vvm aeration rate were 0.775, 0.842, and 0.897 g/L, and the biomass productivities were 0.270, 0.279, and 0.286 g/L/day, respectively. The lipid contents of *Chlorella* sp. TT-1 with 0.1, 0.2, and 0.3 vvm aeration rates were 36.12, 37.61, and 39.90 % respectively, and the result of the experiment was obtained that the biomass production and lipid content in microalgae cells increased with the increasing aeration rate. The experimental result of FAME content showed the contents of FAME compositions slightly increase with high aeration rate, and the variance was 4~5 %.

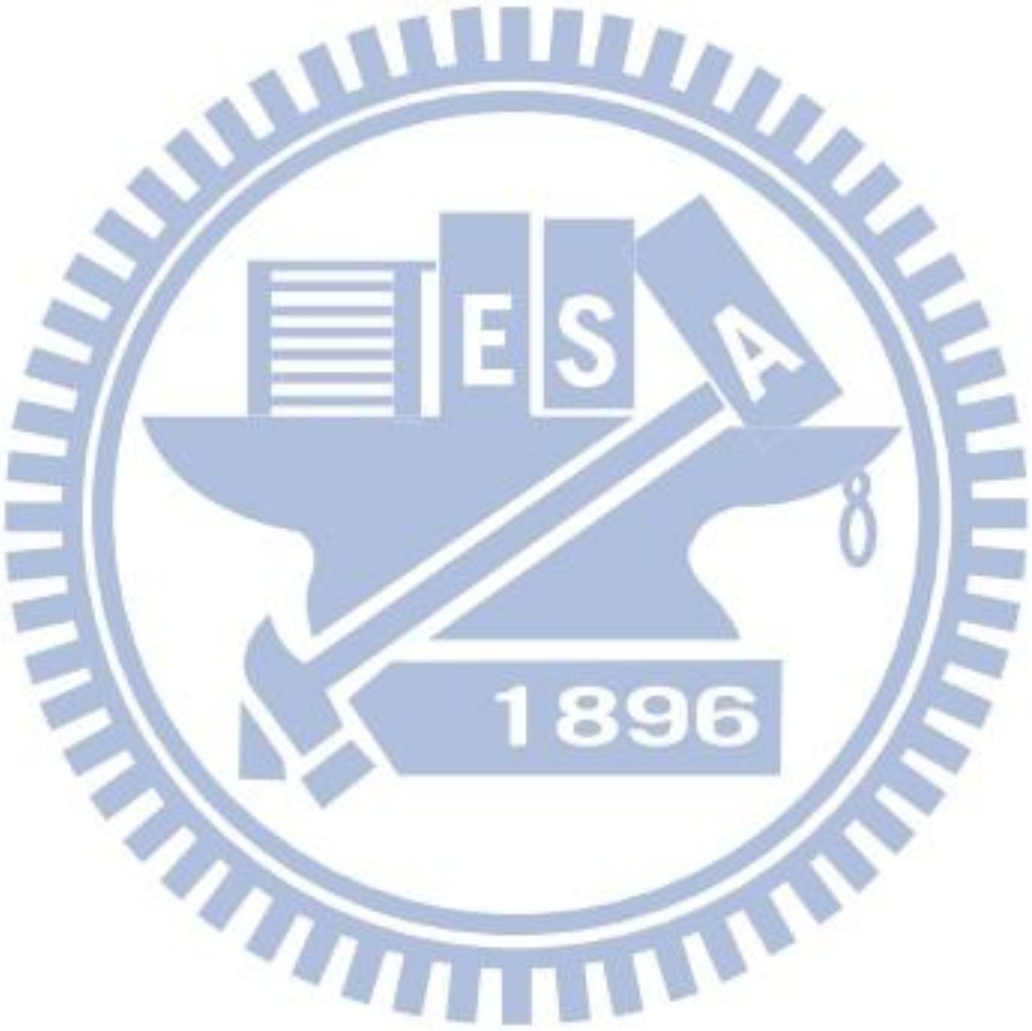
In order to investigate whether the illumination is necessary in microalgae culture aerated with flue gas, *Chlorella* sp. TT-1 was cultivated under full and half illumination. The

biomass production of *Chlorella* sp. TT-1 in full and half illumination were 1.116 g/L and 0.517 g/L respectively. The microalgae cultivation with flue gas under full illumination was obviously better than half illumination, the biomass of *Chlorella* sp. TT-1 cultivated with flue gas under half illumination even reduced during the microalgae aerated with flue gas was shielded from irradiation. In summary, the better irradiation time is full illumination for microalgae cultivation, and the light should be devised when the microalgae cultivated with flue gas. The lipid content of *Chlorella* sp. TT-1 under full and half illumination were 36.90 % and 14.53 %, and the lipid production were 0.412 g/L and 0.083 g/L respectively. Obviously, the lipid in microalgae cell under full illumination was all more than it cultivated under half illumination. The saturated fatty acid (C16:0) accounted for the most part in *Chlorella* sp. TT-1 cultivated under half illumination, while the microalgae had more long and unsaturated fatty acid (C18:1, C18:2, and C18:3) under full illumination.

Instead of enormous experiments to test, we cultivate microalgae *Chlorella* sp. TT-1 combining response surface methodology which can reduce the experiment times to diminish the cost, and simulate the experiment formula to get the optimum condition for microalgae cultivation. In my experiment, initial density, aeration rate, flue gas ratio, and irradiation time these factors were discussed to study the effect to biomass productivity, lipid content, and lipid composition. The optimized biomass productivity of *Chlorella* sp. TT-1 is 0.4864 g/L/day when the initial density of 0.37 g/L aerated with 75 % flue gas ratio at 0.30 vvm aeration rate; the optimized lipid production of *Chlorella* sp. TT-1 is 0.2164 g/L when the initial density of 0.35 g/L aerated with 75 % flue gas ratio at 0.24 vvm aeration rate; the optimized FAME production of *Chlorella* sp. TT-1 is 0.1568 g/L when the initial density of 0.37 g/L aerated with 74 % flue gas ratio at 0.25 vvm aeration rate. The optimum condition was utilized to cultivate microalgae culture in practice, and the predicted value and experimental productivity are shown in **Figure 3-15**.

In order to investigate whether the enhancement of the illumination is necessary in microalgae culture aerated with flue gas under the optimum microalgae cultivation condition and the effect of different illumination density for microalgae cultivation, the illumination of 300, 500, and 700 μmol/m^2/s were utilized and irradiated for the microalgae cultivation with flue gas. The microalgae cultivation cultivated with 500 μmol/m^2/s had the most microalgae biomass production 1.015 g/L while the biomass productivity was 0.390 g/L/day. The illumination of 500 μmol/m^2/s could enhance the lipid accumulation, and it could obtain the

lipid content of 48.68 %. Furthermore, the culture irradiated under 500 μmol/m^2/s had more C16:0 content, and it means higher illumination would induce microalgae to synthesize the saturated carbon compound of shorter chain like C16:0 which high levels of saturated fatty acids tend to increase the stability of FAME.



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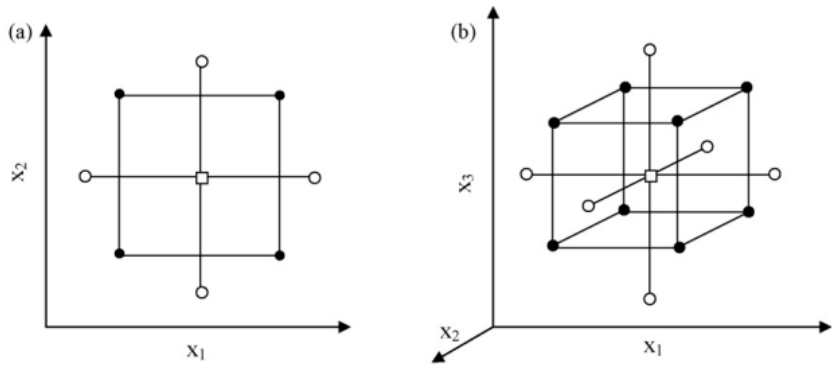

Figure 1-1 Central composite designs for the optimization of: (a) two variables (α = 1.41) and (b) three variables (α = 1.68). ● Points of factorial design, ○ axial points, and □ central point [Bezerra et al., 2008].

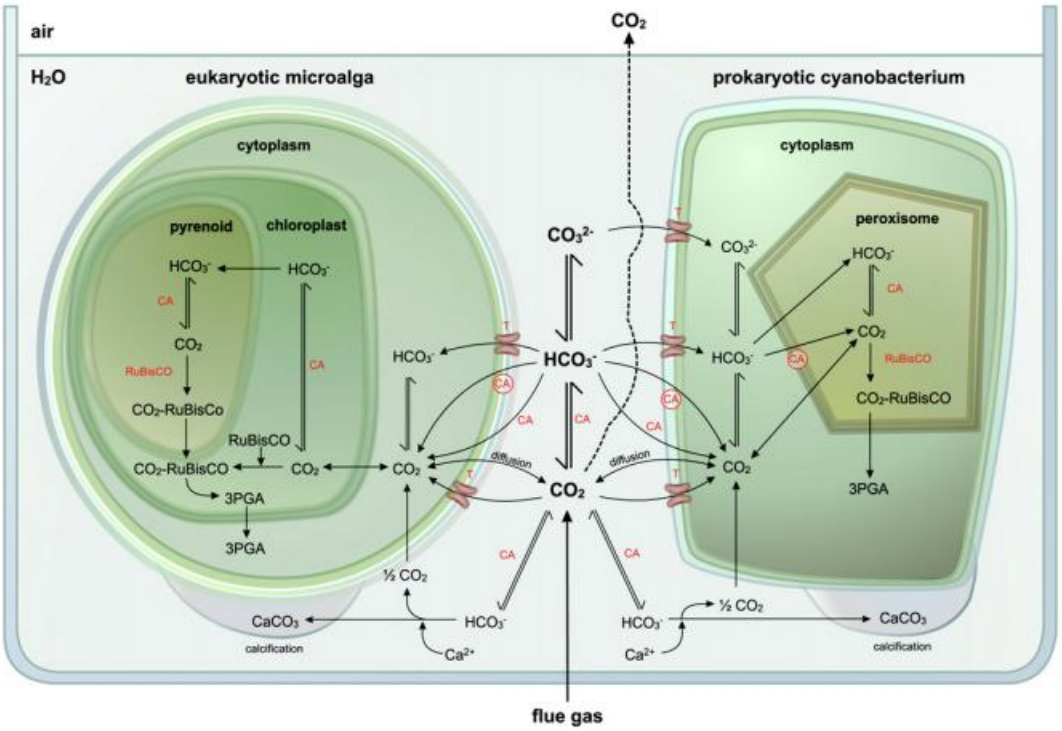

Figure 1-2 Proposed models of inorganic carbon uptake and storage pathways in eukaryotic microalgae and prokaryotic cyanobacteria. CO_2 can diffuse freely across membranes. Since bicarbonate and carbonate are charged ions, their uptake requires transporter molecules (T) and energy. Transformation of extracellular bicarbonate into CO_2 in the periplasmic space is therefore advantageous for the cells, as is cytoplasmic entrapment of CO_2. Similarly, carbonic anhydrase (CA) in chloroplasts (microalgae) and peroxisomes (cyanobacteria) may trap bicarbonate within the organelle and regenerate CO_2 adjacent to rubisco where it is needed for fixation. Carbonate can be stored at the cell wall while producing CO_2 via calcification [Van Den Hende et al., 2012].

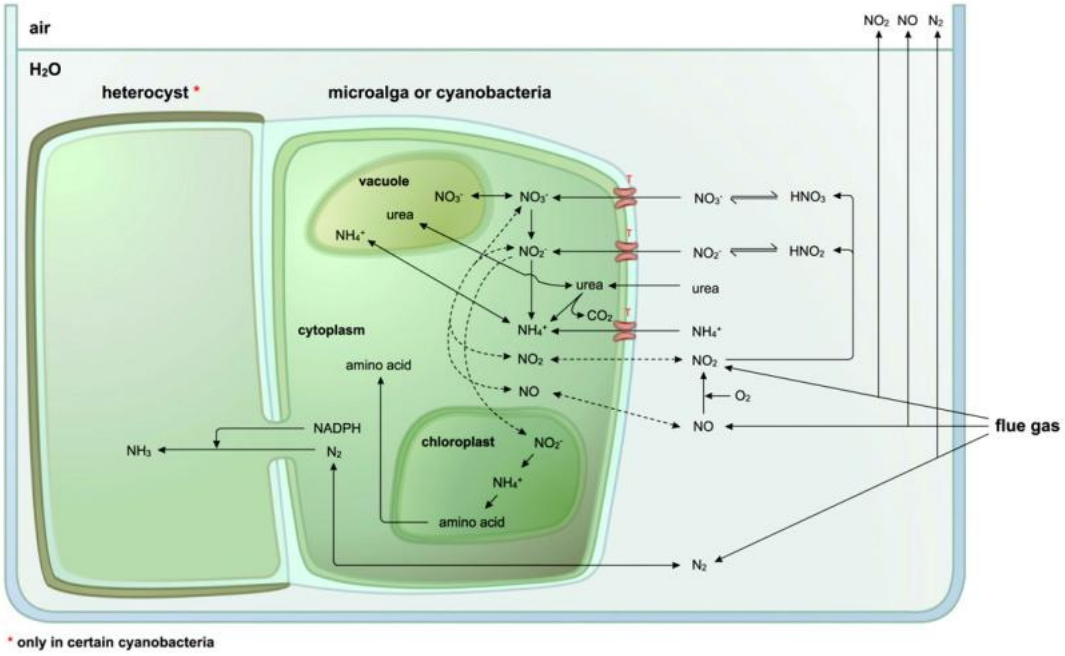

Figure 1-3 Proposed models for nitrogen uptake and storage by eukaryotic microalgae and prokaryotic cyanobacteria. Following uptake by a membrane transport system (T), ammonium can be directly converted into reduced organic nitrogen and utilised in protein production. By contrast, nitrate needs to be reduced to nitrite and ammonium by nitrate reductase. NO is suggested to freely diffuse through the cell membrane and can be assimilated. NO can also be produced by microalgae from nitrite. Certain cyanobacteria can fix N_2 in a heterocyst adjacent to a vegetative cell. Diffusion of N_2 only occurs via vegetative cells, since heterocysts have thick cell walls that prevent O_2 diffusion. Dotted lines indicate pathways which need further investigation [Van Den Hende et al., 2012].

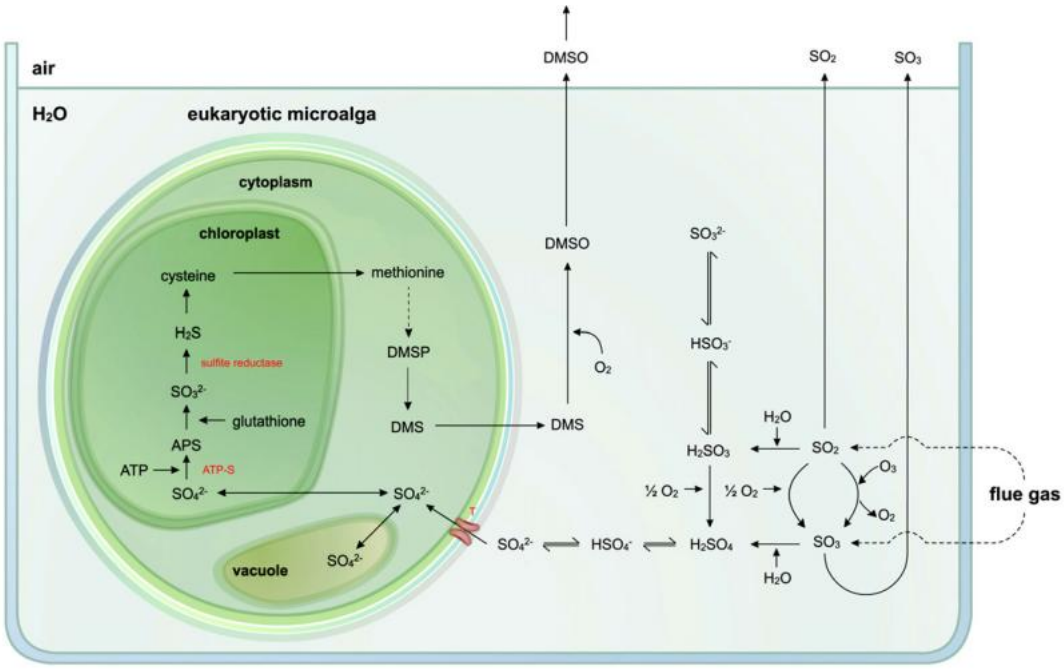

Figure 1-4 Proposed models for sulphur uptake and storage by both prokaryotic cyanobacteria and eukaryotic microalgae. Flue gas compounds SO_2 and SO_3 are converted into sulphate, which is transported into the cell and if present in excess, stored in vacuoles. In the chloroplast, sulphate is reduced to APS, sulphite and sulphide, which is immediately incorporated into cysteine and methionine. Microalgae can excrete DMS, which is oxidised to DMSO and produces sulphate aerosols in the atmosphere.T: transporter; APS: 5'-adenylsulphate; ATP-S: ATP sulphurase; DMSP: dimethylsulphide proprionate; DMS: dimethyl sulphide; DMOS: dimethyl sulphoxide [Van Den Hende et al., 2012].

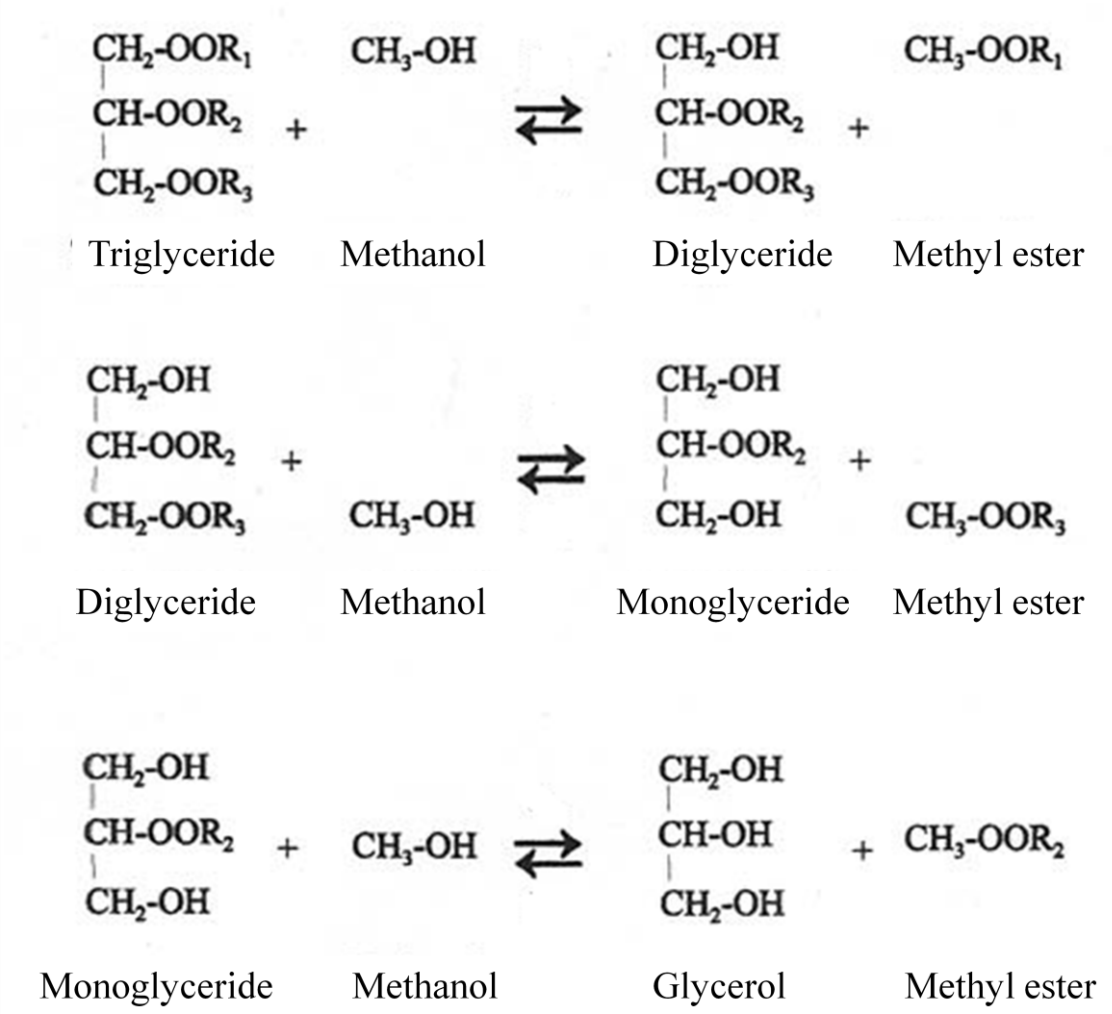

Figure 1-5 Transesterification of triglycerides (overall reaction).

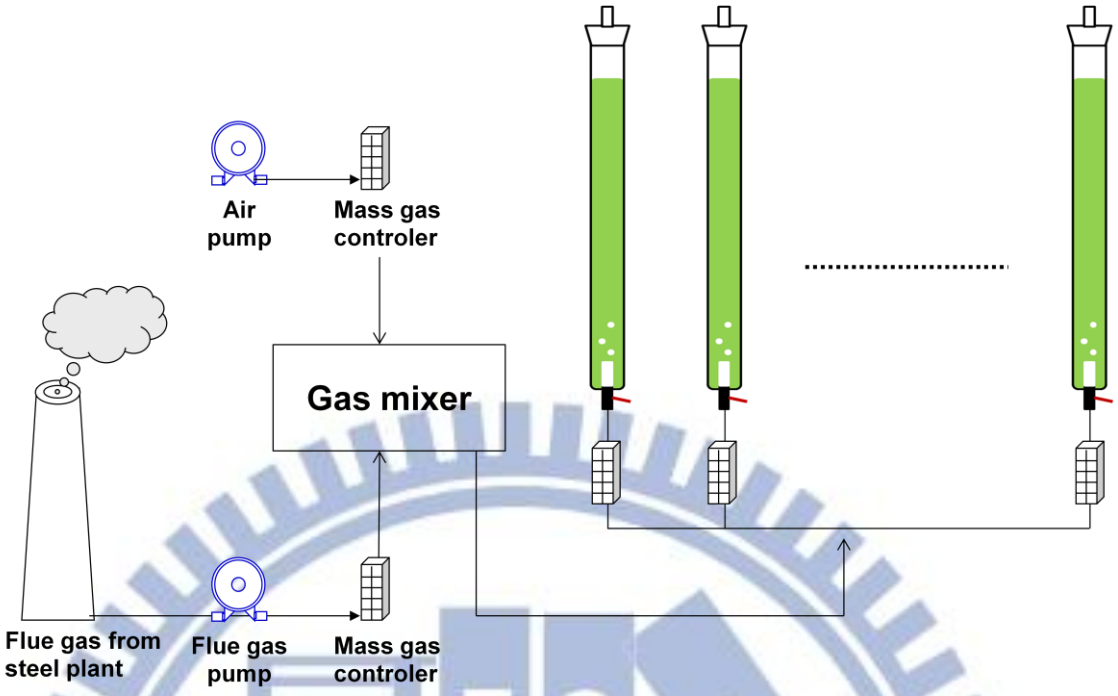

Figure 2-1 Microalgae cultivation system. The photobioreactor was a cylindrical glass column which the diameter and length of the photobioreactor was 5 cm and 80 cm respectively. The gas was supplied from the bottom of the photobioreactor. The flue gas was collected from coke oven in China Steel Corporation, and was exhausted into tank which offered the space to mix it with air. Different flue gas and air volume mixed in the tank would form different ratio gases which would be introduced into bioreactors and utilized by microalgae as nutrient.

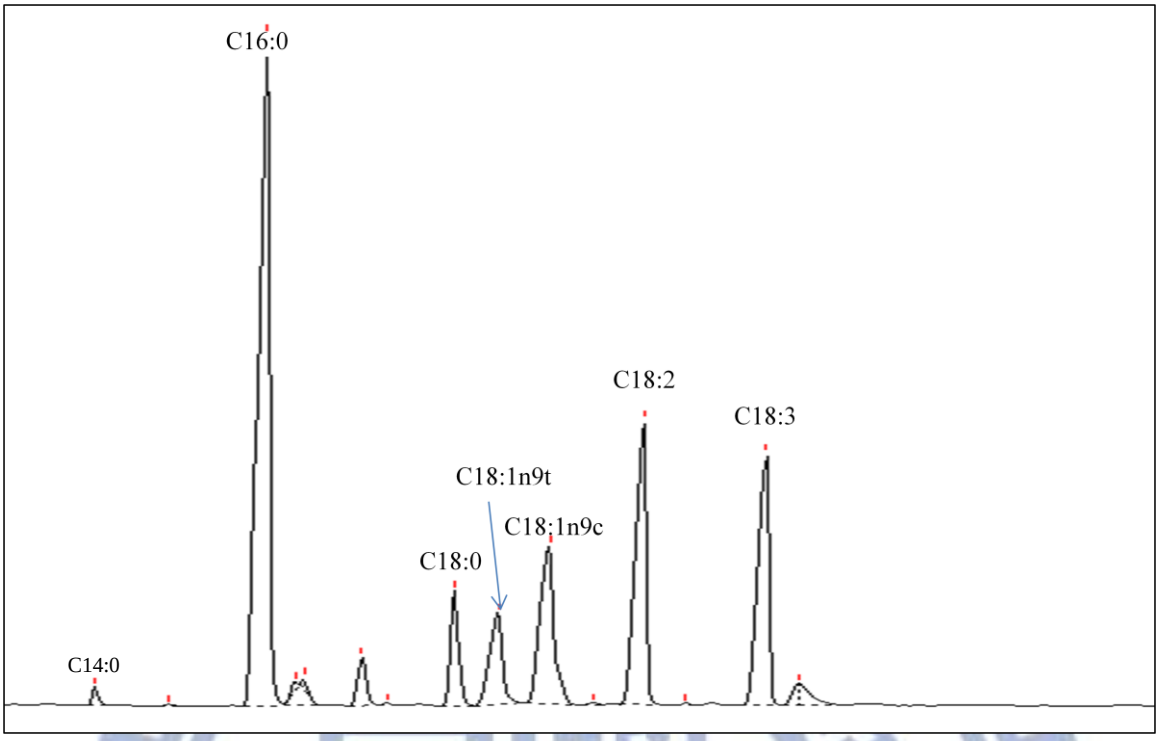

Figure 2-2 The gas chromatography (GC) profile of fatty acid methyl ester (FAME) from microalgae. The lipid extracted from microalgae is transesterificated and produces FAME. The major FAME in microalgae are C_{16} and C_{18}, and especially C16:0, C18:0, C18:1,and C18:2 are appropriated for biodiesel production.

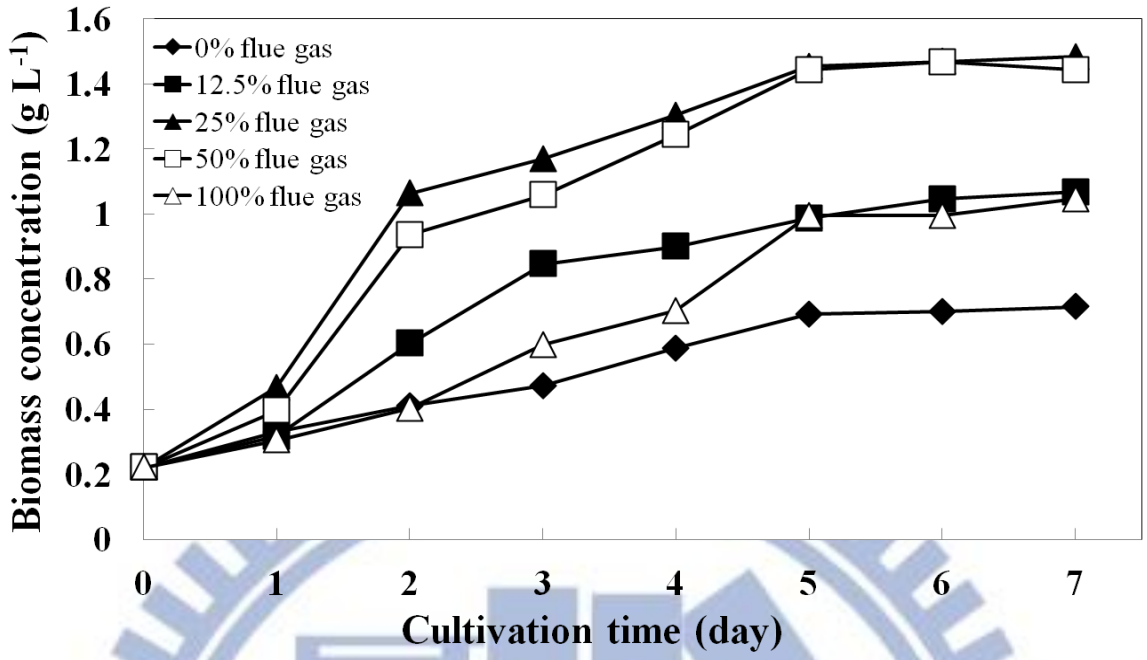

Figure 3-1 Growth profile of *Chlorella* sp. TT-1 aerated with different ratios of flue gas. The initial biomass concentration was approximately 0.2 g/L. The microalgae cells were cultivated at 300 μmol/m^2/s, and culture temperature is 28 ℃. The flue gas was provided at 0.2 vvm. The cultures were grown for 7 days, and the microalgae cells were sampled every 24 hr for growth determinations. Optical density at A_{682} is regarded as the reference parameter of growth.

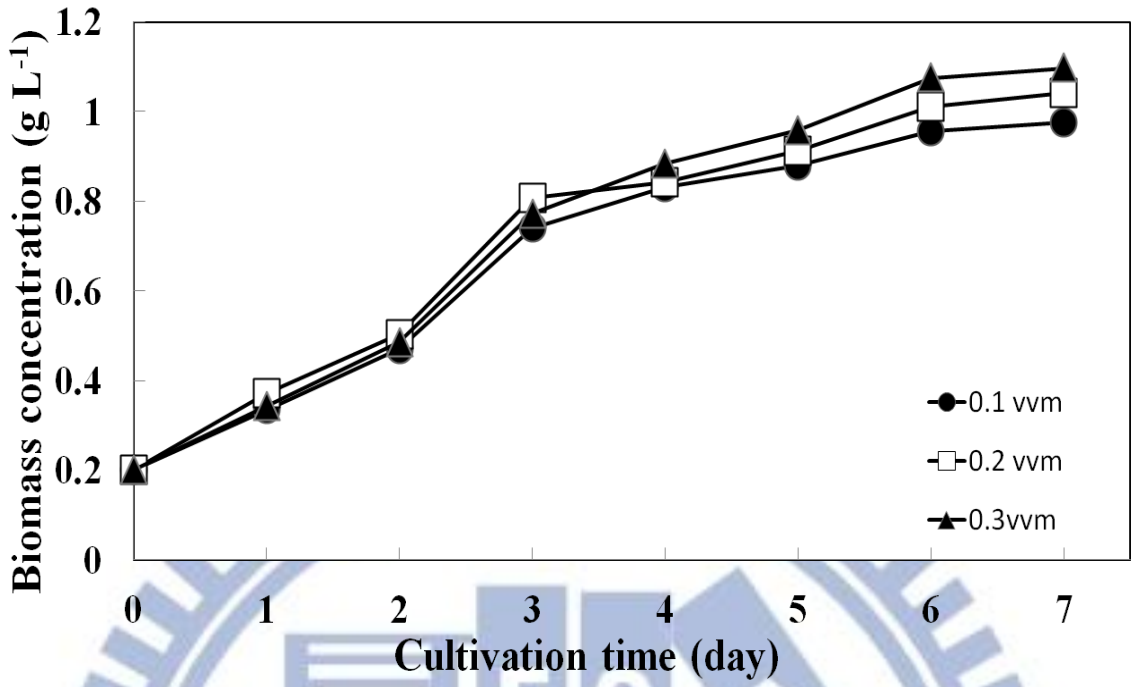

Fig. 3-2 Growth profile of *Chlorella* sp. TT-1 aerated with different aeration rate of flue gas. The initial biomass concentration was approximately 0.2 g/L. The microalgae cells were cultivated at 300 μmol/m^2/s, and culture temperature is 28 ℃. The cultivated air was composed of 25 % flue gas. The cultures were grown for 7 days, and the microalgae cells were sampled every 24 hr for growth determinations. Optical density at A_{682} is regarded as the reference parameter of growth.

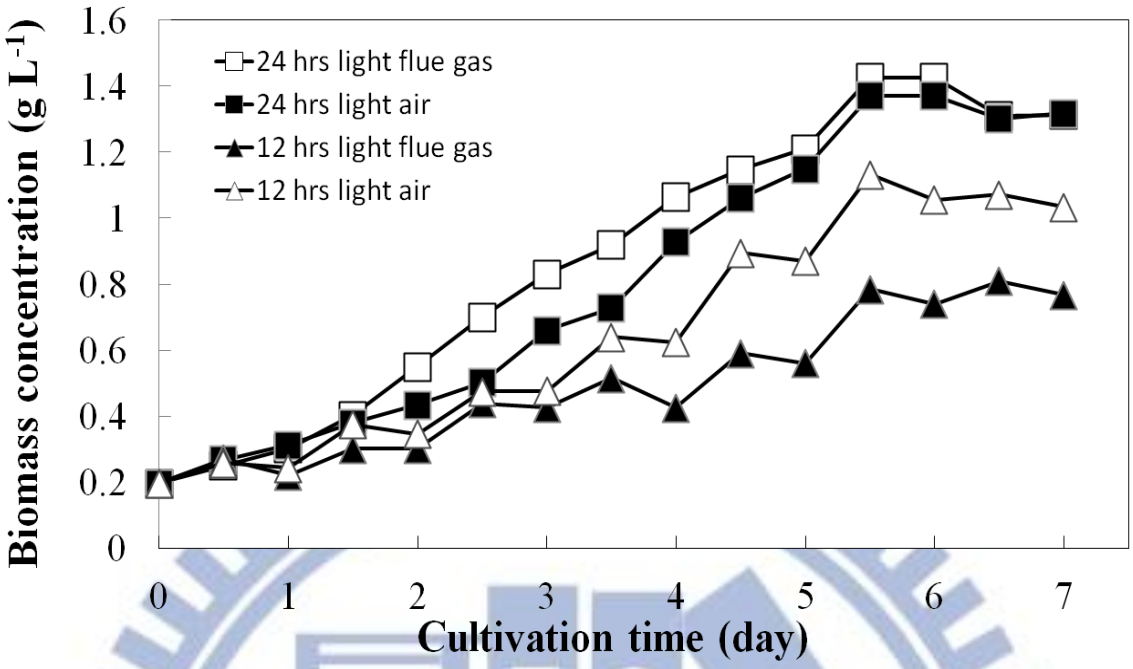

Fig 3-3 Growth profile of *Chlorella* sp. TT-1 aerated with flue gas and air under different illumination time (12 hr and 24 hr). The initial biomass concentration was approximately 0.2 g/L. The microalgae cells were cultivated at 300 μmol/m^2/s. The cultivated air was composed of 25 % flue gas, and the aeration rate used is 0.2 vvm. The cultures were grown for 7 days, and the microalgae cells were sampled every 12 hr for growth determinations.

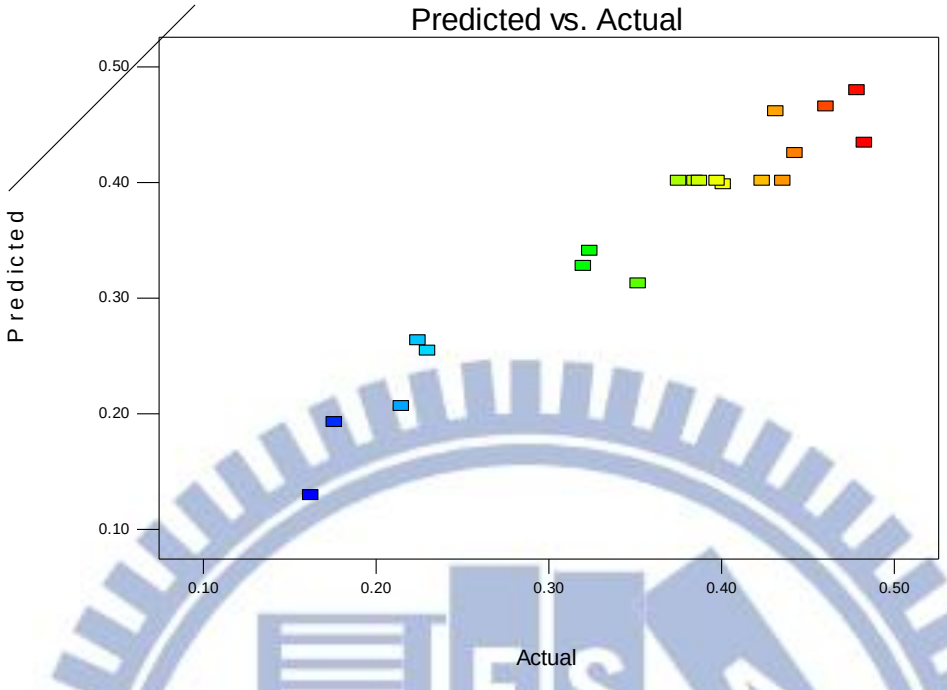

Figure 3-4 The actual biomass productivity and predicted value plot for the microalgae of *Chlorella* sp. TT-1. The plot for biomass productivity which obtained from the experiments was analyzed by using software Design-expert 8.0.6.

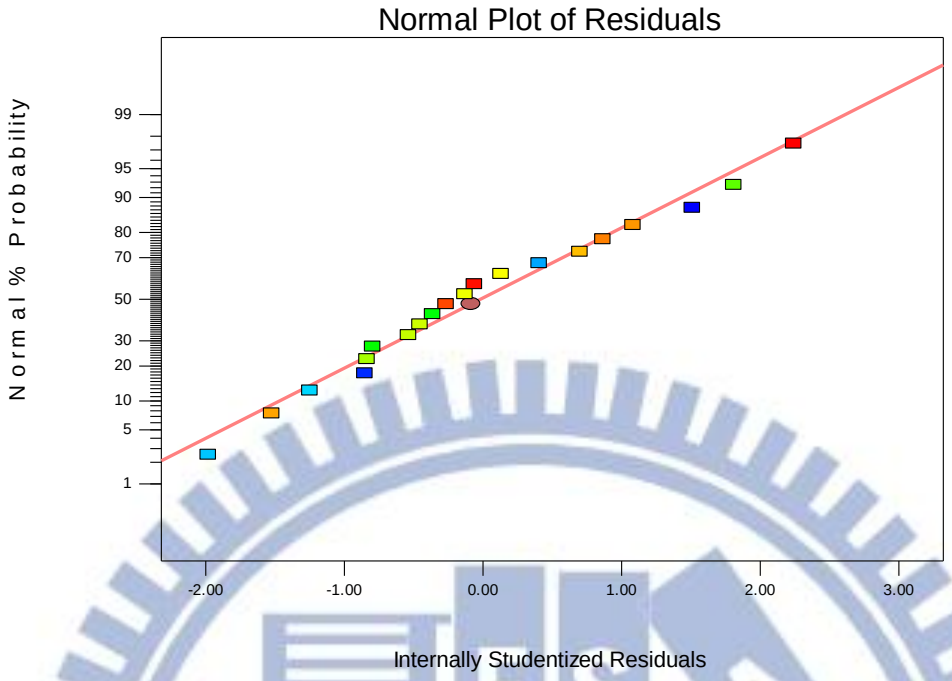

Figure 3-5 The internally studentized residuals and normal % probability plot for the biomass productivity of *Chlorella* sp. TT-1. The plot for biomass productivity which obtained from the experiments was analyzed by using software Design-expert 8.0.6.

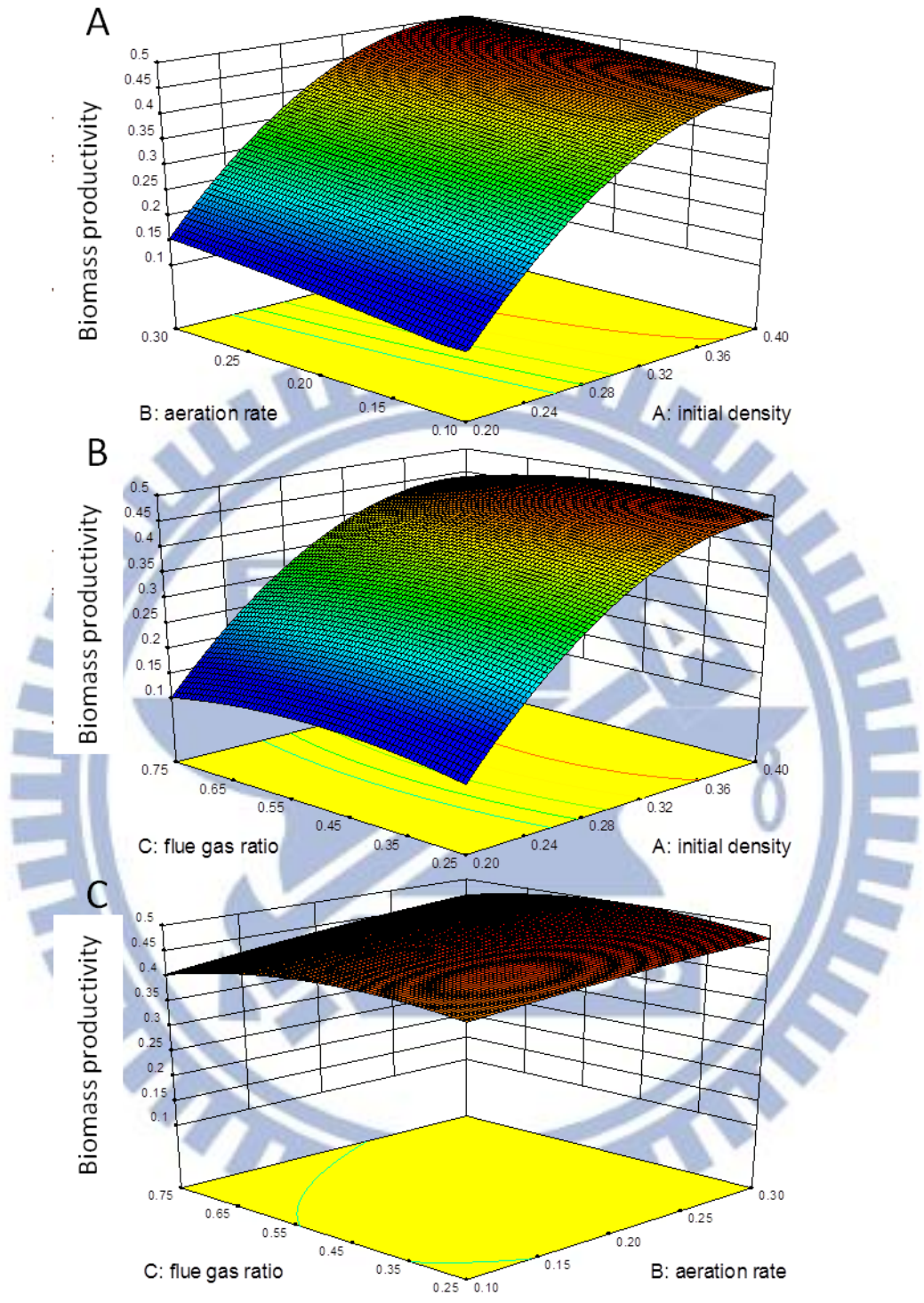

Figure 3-6 The 3D reaction surface plot for the biomass productivity of *Chlorella* sp. TT-1. The microalgae cells were cultivated at 300 μmol/m^2/s, and culture temperature is 28 ℃. The cultures were grown for 7 days, and the microalgae cells were sampled every 24 hr for growth determinations. The 3D reaction surface plot of biomass productivity which obtained from the experiments was analyzed by using software Design-expert 8.0.6.

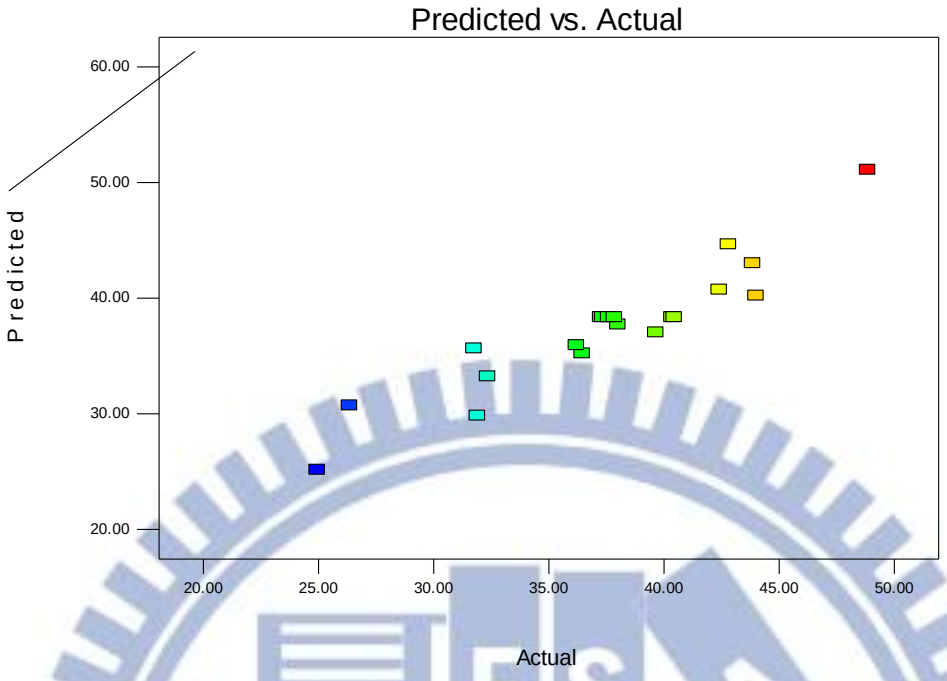

Figure 3-7 The actual lipid content and predicted value plot for the microalgae of *Chlorella* sp. TT-1. The plot for lipid content which obtained from the experiments was analyzed by using software Design-expert 8.0.6.

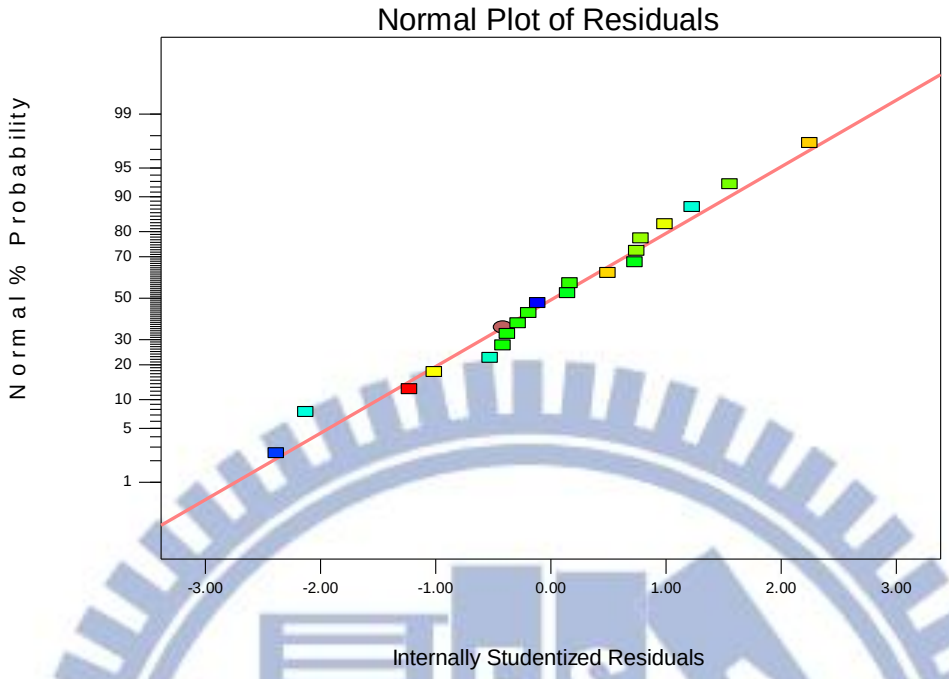

Figure 3-8 The internally studentized residuals and normal % probability plot for the lipid content of *Chlorella* sp. TT-1. The plot for lipid content which obtained from the experiments was analyzed by using software Design-expert 8.0.6.

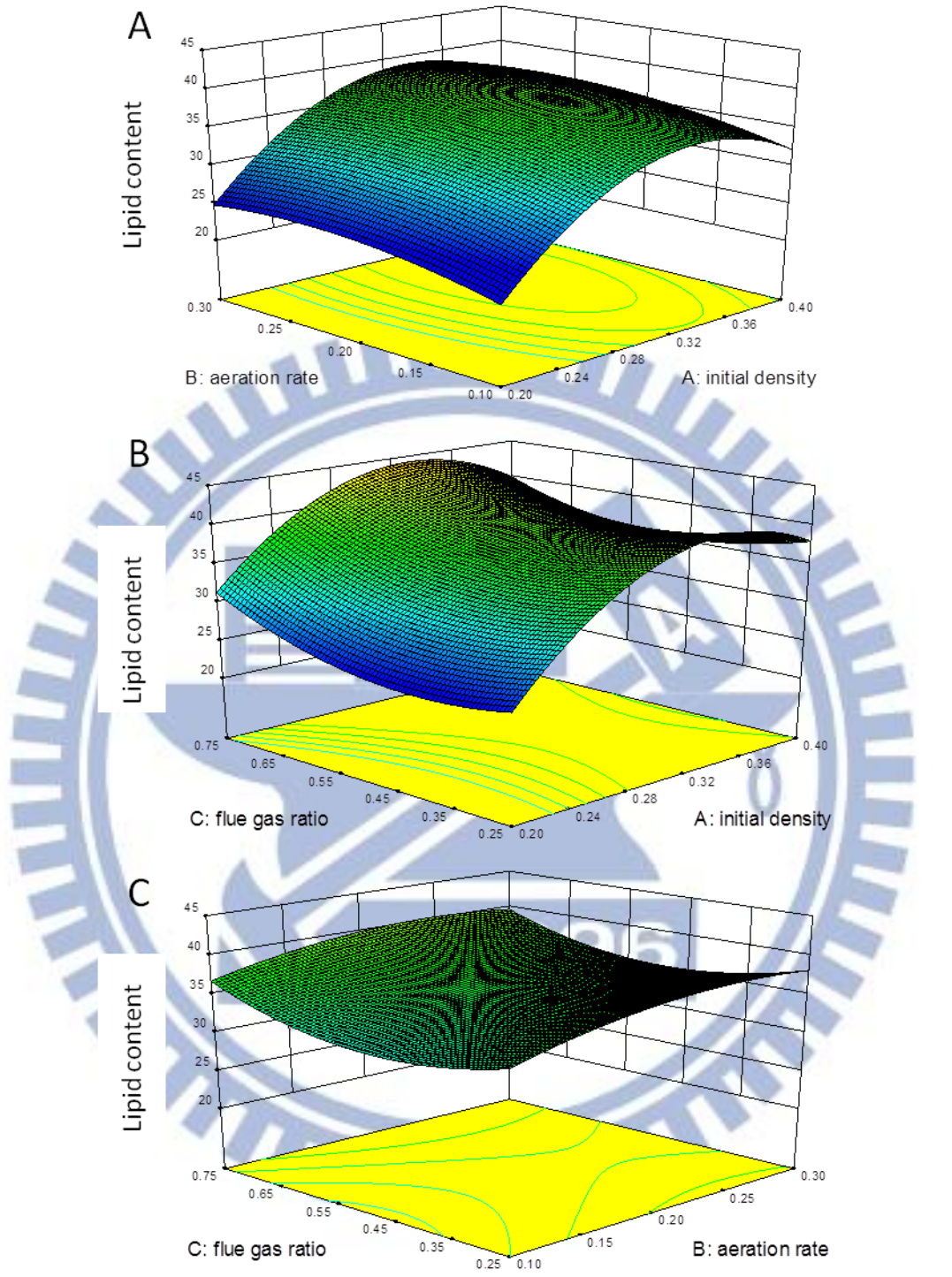

Figure 3-9 The 3D reaction surface plot for the lipid content of *Chlorella* sp. TT-1. The microalgae cells were cultivated at 300 μmol/m^2/s, and culture temperature is 28 ℃. The cultures were grown for 7 days, and *Chlorella* sp. TT-1 at stationary phase was collected after 7 days to measure lipid content. The 3D reaction surface plot of lipid content which obtained from the experiments was analyzed by using software Design-expert 8.0.6.

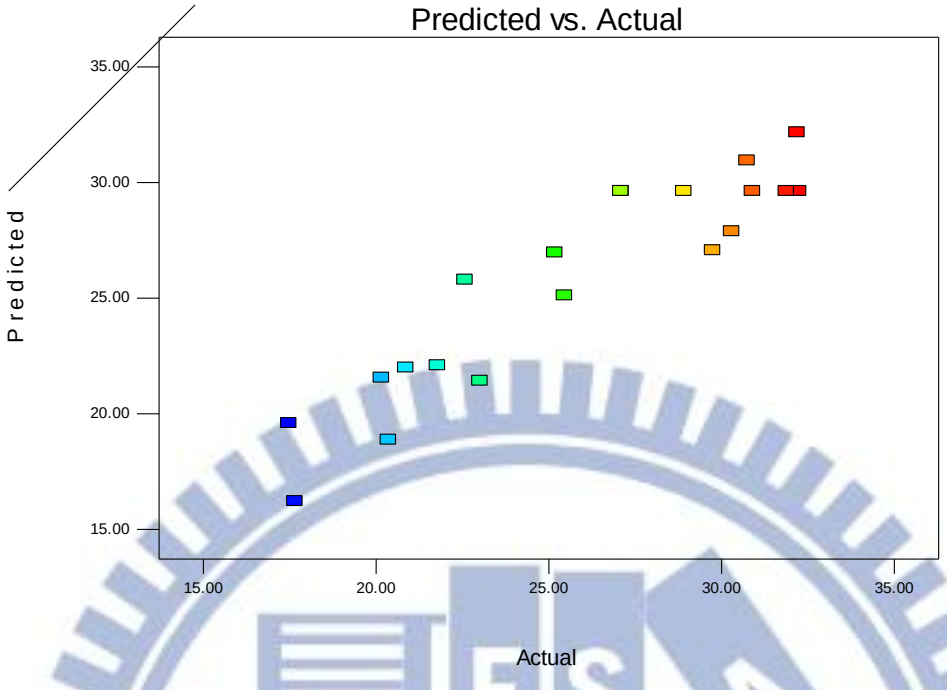

Figure 3-10 The actual FAME content and predicted value plot for the microalgae of *Chlorella* sp. TT-1. The plot for FAME content which obtained from the experiments was analyzed by using software Design-expert 8.0.6.

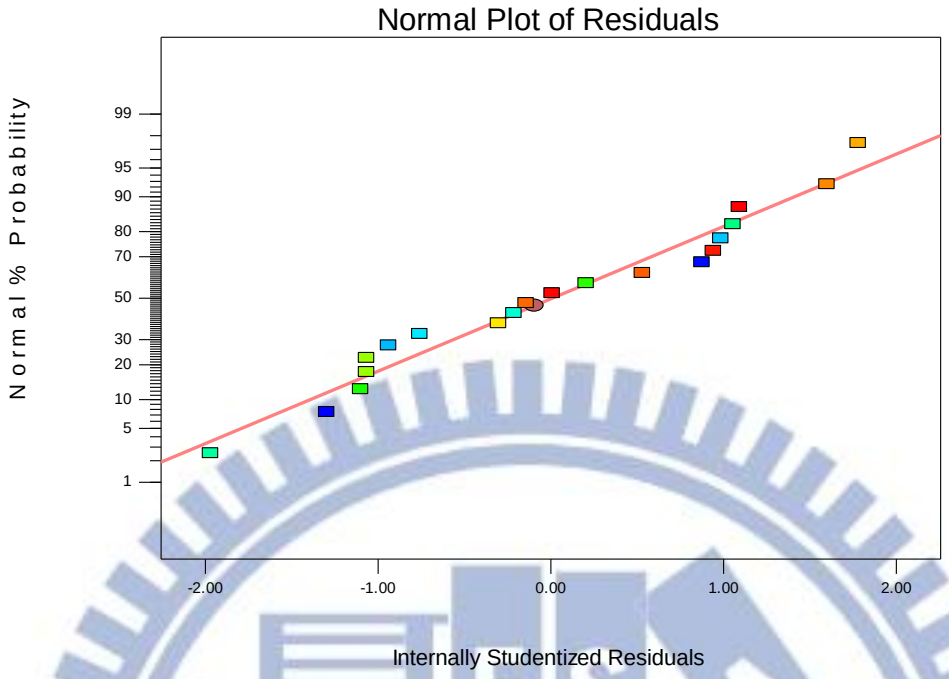

Figure 3-11 The internally studentized residuals and normal % probability plot for the FAME content of *Chlorella* sp. TT-1. The plot for FAME content which obtained from the experiments was analyzed by using software Design-expert 8.0.6.

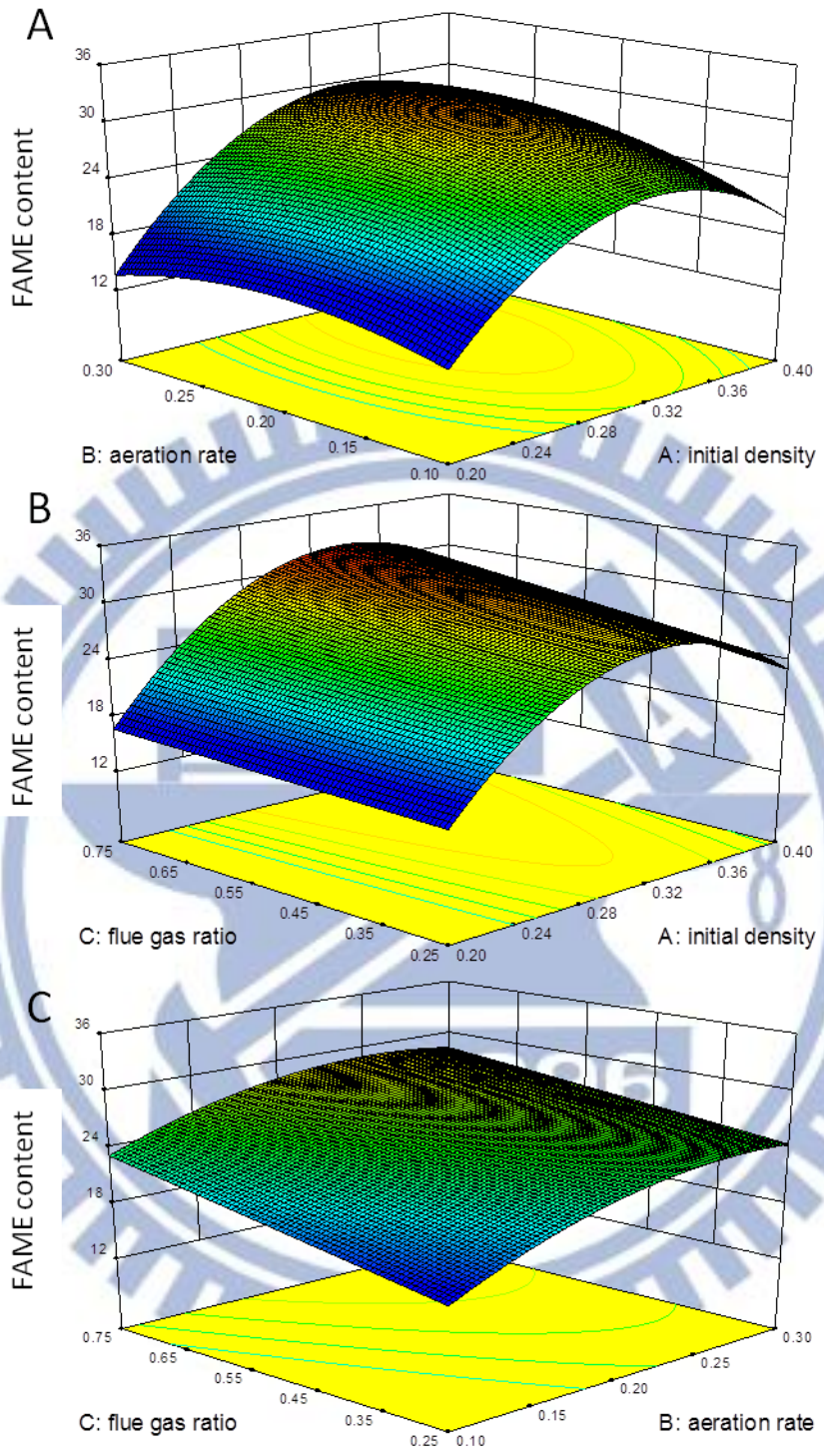

Figure 3-12 The 3D reaction surface plot for the lipid content of *Chlorella* sp. TT-1. The microalgae cells were cultivated at 300 μmol/m^2/s, and culture temperature is 28 ℃. The cultures were grown for 7 days, and lipid compositions of *Chlorella* sp. TT-1 were analyzed by Gas chromatography. The 3D reaction surface plot of biodiesel content which obtained from the experiments was analyzed by using software Design-expert 8.0.6.

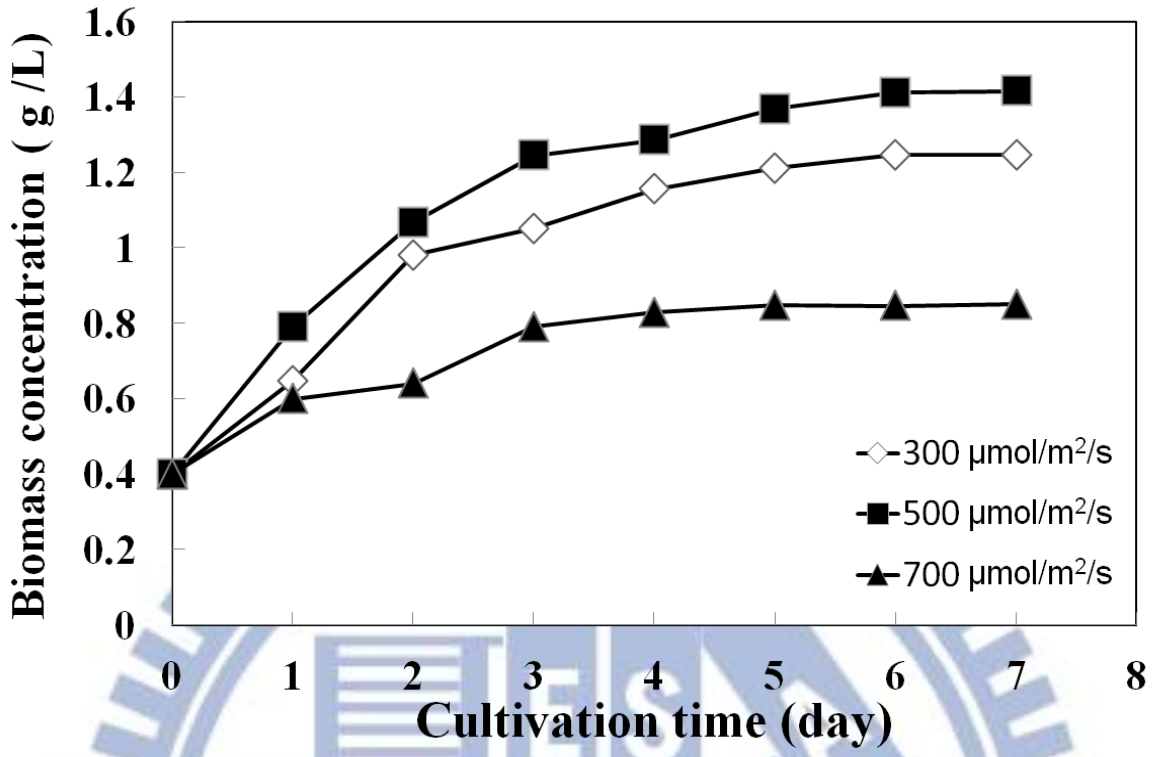

Figure 3-13 Growth profile of *Chlorella* sp. TT-1 aerated with different illuminations. *Chlorella* sp. TT-1 in batch culture was incubated for 7 days at 28 ± 2 ℃. The microalgae was cultivated under the optimum microalgae cultivation condition which the initial density of 0.37 g/L aerated with 75 % flue gas ratio at 0.30 vvm aeration rate. The cultures were sampled when a stationary phase of growth was reached or a microalgae growth was significantly inhibited. Biomass productivity was calculated from the logarithmic growth phase over 1~3 days batch culture in each experiment. Optical density at A_{682} is regarded as the reference parameter of growth.

Table 1-1 Present state of microalgae production

Algae	Annual production	Producer country	Application and product
Arthrospira	3000 t dry weight	China, India, USA, Myanmar, Japan	Human and animal nutrition, cosmetics, phycobiliproteins
Chlorella	2000 t dry weight	Taiwan, Germany, Japan	Human nutrition, aquaculture, cosmetics
Dunaliella salina	1200 t dry weight	Australia, Israel, USA, China	Human nutrition, cosmetics, β-carotene
Aphanizomenon flos-aquae	500 t dry weight	USA	Human nutrition
Haematococcus pluvialis	300 t dry weight	USA, India, Israel	Aquaculture, astaxanthin
Crypthecodinium cohnii	240 t DHA oil	USA	DHA oil
Shizochytrium	10 t DHA oil	USA	DHA oil
Spirulina	3000 t dry weight	China, India, USA, Myanmar, Japan	Human and Animal nutrition Cosmetics Phycobiliproteins

Table 1-2 Temperature and flue gas tolerance of various algal species

Algal species	Maximum temperature tolerance (℃)	Maximum CO_2 % tolerance	Maximum SO_x (ppm) tolerance	Maximum NO_x (ppm) tolerance	References
Cyanidium caldarium	60	100			Seckbach et al., 1972
Scenedesmus sp.	30	80			Hanagata et al., 1992
Chlorococcum littorale		70			Ota et al. 2009
Synechococcus elongates	60	60			Miyairi. 1995
Euglena gracilis		45			Nakano et al., 1996
***Chlorella* sp.**	45	40			Hanagata et al., 1992
***Chlorella* sp. HA–1**		15		100	Yanagi et al., 1995
***Eudorina* sp.**	30	20			Hanagata et al.,1992
Dunaliella tertiolecta		15		1000	Nagase et al., 1998
***Chlamydomonas* sp. MGA 161**	35	15			Miura et al., 1993
***Nannochloris* sp.**	25	15		100	Yoshihara et al., 1996
***Tetraselmis* sp.**		14	185	125	Matsumoto et al., 1995
Monoraphidium minutum	25	13.6	200	150	Zeiler et al., 1995
***Spirulina* sp.**		12			de Morais and Costa, 2007
***Chlorella* sp. T-1**	35		20	60	Maeda et al., 1995

Table 1-3 Experimental matrices for central composite design: (a) two variables and (b) three variables

Two variables	x_1	x_2
Factorial design	-1	-1
	1	-1
	-1	1
	1	1
Axial points	-α	0
	α	0
	0	α
	0	-α
Central point	0	0

Three variables	x_1	x_2	x_3
Factorial design	-1	-1	-1
	1	-1	-1
	-1	1	-1
	1	1	-1
	-1	-1	1
	1	-1	1
	-1	1	1
	1	1	1
Axial points	-α	0	0
	α	0	0
	0	-α	0
	0	α	0
	0	0	-α
	0	0	α
Central point	0	0	0

Table 1-4 Comparison of microalgae with other biodiesel feedstocks.

Plant source	Seed oil content (% oil by wt in biomass)	Oil yield (L oil/ha year)	Land use (m^2 year/kg biodiesel)	Biodiesel productivity (kg biodiesel/ha year)
Corn/Maize	44	172	66	152
Hemp	33	363	31	321
Soybean	18	636	18	562
Jatropha	28	741	15	656
Camelina	42	915	12	809
Canola/Rapeseed	41	974	12	862
Sunflower	40	1070	11	946
Castor	48	1307	9	1156
Paim oil	36	5366	2	4747
Microalgae (low oil content)	30	58700	0.2	51927
Microalgae (medium oil content)	50	97800	0.1	86515
Microalgae (high oil content)	70	136900	0.1	121104

Table 1-5 Comparison of properties of biodiesel, diesel fuel and ASTM standard

Properties	Biodiesel from microalgae oil	Diesel fuel	ASTM biodiesel standard
Density (kg/l)	0.864	0.838	0.86 ~ 0.90
viscosity (mm^2/s, cst at 40℃)	5.2	1.9~4.1	3.5 ~ 5.0
Flash point (℃)	115	75	min 100
Solifiying point (℃)	-12	-50 to 10	-
Cold filter plugging point (℃)	-11	3.0 (max -6.7)	summer max 0 winner max <-15
Acid value (mg KOH/g)	0.374	max 0.5	max 0.5
Heat value (Mj/kg)	41	40 ~ 50	-
H/C ratio	1.81	1.81	-

Table 3-1 Biomass and lipid production of *Chlorella* sp. TT-1 aerated with different ratios of flue gas. [a]

Flue gas ratio (%)	Biomass production (g/L)	Lipid content (%)	Lipid production (g/L) [b]
0	0.515 ± 0.127	32.18 ± 0.13	0.166 ± 0.025
12.5	0.867 ± 0.041	41.55 ± 0.43	0.360 ± 0.017
25	1.284 ± 0.015	40.23 ± 0.68	0.517 ± 0.109
50	1.243 ± 0.000	36.53 ± 1.30	0.454 ± 0.088
100	0.847 ± 0.026	36.93 ± 0.43	0.313 ± 0.070

a. The initial biomass concentration was approximately 0.2 g/L. The microalgae cells were cultivated at 300 μmol/m^2/s, and the culture temperature was 28 ℃. The flue gas was provided at 0.2 vvm. The cultures were grown for 7 days, and *Chlorella* sp. TT-1 at stationary phase was collected after 7 days to measure lipid content.

b. Lipid production (g/L) = (biomass production × lipid content) / 100

c. Each data indicates the mean ± SD from three experiments.

Table 3-2 Lipid composition profile of *Chlorella* sp. TT-1 aerated with different ratios of flue gas. [a]

	Flue gas ratio				
	1	0.5	0.25	0.125	0
Fatty acid composition [b, c]	**Relative content (%)**				
C16:0	39.67±0.30	39.29±0.08	39.09±0.30	40.12±1.83	41.00±0.41
C16:1	0.78±0.00	0.88±0.04	1.45±0.02	1.19±0.41	0.38±0.21
C18:0	5.37±0.06	5.54±0.11	5.97±0.10	5.53±0.08	4.27±0.01
C18:1	11.65±0.07	12.05±0.17	11.20±0.21	11.68±0.28	9.18±0.08
C18:2	16.29±0.08	16.72±0.08	16.21±0.30	15.86±0.24	14.51±0.10
C18:3	16.39±0.24	16.25±0.16	15.40±0.84	15.47±0.29	19.48±0.23
others	9.85±0.15	9.26±0.39	10.69±0.08	10.15±0.53	11.18±0.60

a. The initial biomass concentration was approximately 0.2 g/L. The microalgae cells were cultivated at 300 μmol/m^2/s, and the culture temperature was 28 ℃. The flue gas was provided at 0.2 vvm. The cultures were grown for 7 days, and lipid compositions of *Chlorella* sp. TT-1 were analyzed by Gas chromatography.

b. Lipid compositions were analyzed by Gas chromatography.

c. The appropriate lipid composition for FAME are palmitic acid (C16:0), Stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), and α-Linolenic acid (C18:3).

Table 3-3 Biomass and lipid production of *Chlorella* sp. TT-1 aerated at different aeration rates. [a]

Aeration rate (vvm)	Biomass production (g/L)	Lipid content (%)	Lipid production (g/L) [b]
0.1	0.775 ± 0.014	36.12 ± 0.28	0.280 ± 0.005
0.2	0.842 ± 0.003	37.62 ± 0.28	0.317 ± 0.054
0.3	0.897 ± 0.029	39.90 ± 0.25	0.358 ± 0.019

a. The initial biomass concentration was approximately 0.2 g/L. The microalgae cells were cultivated at 300 μmol/m^2/s, and the culture temperature was 28 ℃. The cultivated air was composed of 25 % flue gas. The cultures were grown for 7 days, and *Chlorella* sp. TT-1 at stationary phase was collected after 7 days to measure lipid content.

b. Lipid production (g/L) = (biomass production × lipid content) / 100

c. Each data indicates the mean ± SD from three experiments.

Table 3-4 Lipid composition profile of *Chlorella* sp. TT-1 aerated at different aeration rates. [a]

Aeration rate	0.1 vvm	0.2 vvm	0.3 vvm
Fatty acid composition [b, c]	**Relative content (%)**		
C16:0	35.73 ± 0.13	36.07 ± 0.04	37.19 ± 0.27
C16:1	1.22 ± 0.01	1.37 ± 0.01	1.16 ± 0.00
C18:0	4.75 ± 0.12	4.84 ± 0.05	4.54 ± 0.06
C18:1	7.93 ± 0.03	8.14 ± 0.11	8.58 ± 0.06
C18:2	18.22 ± 0.11	18.19 ± 0.09	18.43 ± 0.26
C18:3	20.18 ± 0.09	18.96 ± 0.11	18.25 ± 0.40
others	11.96 ± 0.02	12.43 ± 0.31	11.86 ± 0.40

a. The initial biomass concentration was approximately 0.2 g/L. The microalgae cells were cultivated at 300 μmol/m^2/s, and the culture temperature was 28 ℃. The cultivated air was composed of 25 % flue gas. The cultures were grown for 7 days, and lipid compositions of *Chlorella* sp. TT-1 were analyzed by Gas chromatography.

b. Lipid compositions were analyzed by Gas chromatography.

c. The appropriate lipid composition for FAME are palmitic acid (C16:0), Stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), and α-Linolenic acid (C18:3).

Table 3-5 Biomass and lipid production of *Chlorella* sp. TT-1 cultivated under full and half illumination. [a]

Illumination mode		Biomass production (g/L)	Lipid content (%)	Lipid production (g/L) [b]
24hr	**flue gas**	1.116 ± 0.103	36.90 ± 0.10	0.412 ± 0.023
	air	1.122 ± 0.005	26.23 ± 0.33	0.294 ± 0.015
12hr	**flue gas**	0.571 ± 0.087	14.53 ± 0.23	0.083 ± 0.009
	air	0.837 ± 0.055	14.23 ± 0.03	0.119 ± 0.016

a. The initial biomass concentration was approximately 0.2 g/L. The microalgae cells were cultivated at 300 μmol/m^2/s, and the culture temperature was 28 ℃. The cultivated air was composed of 25 % flue gas, and the aeration rate was 0.2 vvm. The cultures were grown for 7 days, and *Chlorella* sp. TT-1 at stationary phase was collected after 7 days to measure lipid content.

b. Lipid production (g/L) = (biomass production × lipid content) / 100

c. Each data indicates the mean ± SD from three experiments.

Table 3-6 Lipid composition profile of *Chlorella* sp. TT-1 aerated at different aeration rates. [a]

	24 hr		12 hr	
	flue gas	**air**	**flue gas**	**air**
Fatty acid composition [b, c]	**Relative content (%)**			
C16:0	37.95±0.08	25.33±0.23	55.03±0.21	52.49±0.40
C18:0	4.63±0.23	3.94±0.03	4.73±0.01	9.30±0.13
C18:1	12.15±0.01	10.66±0.08	5.44±0.01	16.00±0.27
C18:2	17.04±0.10	14.45±0.26	9.62±0.13	3.18±0.12
C18:3	15.65±0.13	14.46±0.09	11.75±0.40	10.04±0.06
others	12.59±0.03	31.17±0.31	13.43±0.28	8.98±0.02

a. The initial biomass concentration was approximately 0.2 g/L. The microalgae cells were cultivated at 300 μmol/m^2/s, and the culture temperature was 28 ℃. The cultivated air was composed of 25 % flue gas, and the aeration rate is 0.2 vvm. The cultures were grown for 7 days, and lipid compositions of *Chlorella* sp. TT-1 were analyzed by Gas chromatography.

b. Lipid compositions were analyzed by Gas chromatography.

c. The appropriate lipid composition for FAME are palmitic acid (C16:0), Stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), and α-Linolenic acid (C18:3).

Table 3-7 Experimental factors and the values of each level for the optimization of *Chlorella* sp. TT-1 in biomass productivity, lipid content, and FAME content

Factor	-α	-1	0	1	+α
Aeration rate (vvm)	0.03	0.10	0.20	0.30	0.37
Flue gas ratio (%) [a]	0.08	0.25	0.50	0.75	0.92
Initial density (g/L) [b]	0.13	0.20	0.30	0.40	0.47

a. The CO_2 concentration in flue gas is 28 %.

b. Optical density at A_{682} is regarded as the reference parameter of growth.

Table 3-8 Experiment layout designed by Design-Expert 8.0.6 and the results expressing the biomass productivity, lipid content, and FAME content of *Chlorella* sp. TT-1 in different conditions.

Run	Factor 1 Initial density (g/L)	Factor 2 Aeration rate (vvm)	Factor 3 Flue gas ratio (%)	Biomass productivity (g/L/day)	Lipid content (%)	FAME content (%)
1	0.20(-1)	0.10(-1)	0.25(-1)	0.215	31.9	20.36
2	0.40(+1)	0.10(-1)	0.25(-1)	0.443	39.65	23.01
3	0.20(-1)	0.30(+1)	0.25(-1)	0.230	32.35	20.15
4	0.40(+1)	0.30(+1)	0.25(-1)	0.461	42.4	30.29
5	0.20(-1)	0.10(-1)	0.75(+1)	0.176	36.45	20.86
6	0.40(+1)	0.10(-1)	0.75(+1)	0.401	44	29.74
7	0.20(-1)	0.30(+1)	0.75(+1)	0.224	38	21.78
8	0.40(+1)	0.30(+1)	0.75(+1)	0.432	43.85	30.74
9	0.13(-α)	0.20(0)	0.50(0)	0.162	24.95	17.66
10	0.47(+α)	0.20(0)	0.50(0)	0.479	31.75	22.58
11	0.30(0)	0.03(-α)	0.50(0)	0.324	26.35	17.48
12	0.30(0)	0.37(+α)	0.50(0)	0.483	36.2	25.45
13	0.30(0)	0.20(0)	0.08(-α)	0.320	42.8	25.18
14	0.30(0)	0.20(0)	0.92(+α)	0.352	48.85	32.19
15	0.30(0)	0.20(0)	0.50(0)	0.385	40.35	27.09
16	0.30(0)	0.20(0)	0.50(0)	0.387	40.45	28.91
17	0.30(0)	0.20(0)	0.50(0)	0.375	37.25	27.09
18	0.30(0)	0.20(0)	0.50(0)	0.397	37.35	32.24
19	0.30(0)	0.20(0)	0.50(0)	0.424	37.6	31.88
20	0.30(0)	0.20(0)	0.50(0)	0.436	37.85	30.90

Table 3-9 ANOVA table about the biomass productivity of *Chlorella* sp. TT-1 for the quadratic model. [a]

Source	Sum of squares	DF	Mean square	F value	*p*-value
Model [b]	0.1857	9	0.0206	17.4050	< 0.0001
Residual	0.0119	10	0.0012		
Lack of Fit	0.0090	5	0.0018	3.1812	0.1149
Pure Error	0.0028	5	0.0006		
Cor Total	0.1975	19			

a. The table for biomass productivity which obtained from the experiments was analyzed by using software Design-expert 8.0.6.

b. $R^2 = 0.9400$; adjusted $R^2 = 0.8860$

Table 3-10 Parameter values found for the quadratic model about the biomass productivity of *Chlorella* sp. TT-1 and their significances. [a]

Factor	Coefficient estimate	Standard error	Value	p-value
A-initial density	-3.5938	1.0911	3.2036	0.0081
B-aeration rate	0.0050	0.1464	0.4170	0.9737
C-flue gas ratio	-0.0439	0.1464	0.4353	0.7702
AB	-0.0095	0.0609	-0.1902	0.8789
AC	-0.0164	0.0609	-0.1314	0.7927
BC	0.0057	0.0122	0.2283	0.6492
A^2	-0.8573	0.2271	-3.4291	0.0036
B^2	-0.0049	0.0091	-0.4907	0.6003
C^2	-0.0287	0.0091	-0.4596	0.0100

a. The table for biomass productivity which obtained from the experiments was analyzed by using software Design-expert 8.0.6.

Table 3-11 ANOVA table about the lipid content of *Chlorella* sp. TT-1 for the quadratic model. [a]

Source	Sum of squares	DF	Mean square	F value	*p*-value
Model [b]	575.4417	9	63.9380	7.4760	0.0021
Residual	85.5247	10	8.5525		
Lack of Fit	74.1859	5	14.8372	6.5427	0.0300
Pure Error	11.3388	5	2.2678		
Cor Total	660.9664	19			

a. The table for lipid content which obtained from the experiments was analyzed by using software Design-expert 8.0.6.

b. $R^2 = 0.8705$; adjusted $R^2 = 0.7539$

Table 3-12 Parameter values found for the quadratic model about the lipid content of *Chlorella* sp. TT-1 and their significances. [a]

Factor	Coefficient estimate	Standard error	Value	p-value
A-initial density	-322.4720	92.7365	209.7839	0.0059
B-aeration rate	2.4498	12.4398	88.9013	0.8478
C-flue gas ratio	-4.6834	12.4398	-37.7796	0.7144
AB	0.3750	5.1727	7.5000	0.9436
AC	-2.7500	5.1727	-22.0000	0.6066
BC	-0.2250	1.0345	-9.0000	0.8322
A^2	-70.4357	19.3025	-281.7427	0.0045
B^2	-1.7788	0.7708	-177.8827	0.0437
C^2	3.3654	0.7708	53.8460	0.0014

a. The table for lipid content which obtained from the experiments was analyzed by using software Design-expert 8.0.6.

Table 3-13 ANOVA table about the FAME content of *Chlorella* sp. TT-1 for the quadratic model. [a]

Source	Sum of squares	DF	Mean square	F value	*p*-value
Model [b]	406.4463	9	45.1607	6.6243	0.0034
Residual	68.1739	10	6.8174		
Lack of Fit	41.3346	5	8.2669	1.5401	0.3236
Pure Error	26.8393	5	5.3679		
Cor Total	474.6202	19			

a. The table for FAME content which obtained from the experiments was analyzed by using software Design-expert 8.0.6.

b. $R^2 = 0.8564$; adjusted $R^2 = 0.7271$

Table 3-14 Parameter values found for the quadratic model about the FAME content of *Chlorella* sp. TT-1 and their significances. [a]

Factor	Coefficient estimate	Standard error	Value	p-value
A-initial density	-352.2630	82.7496	180.2154	0.0017
B-aeration rate	12.9942	11.1001	103.8101	0.2689
C-flue gas ratio	9.1251	11.1001	4.0944	0.4302
AB	4.7306	4.6157	94.6119	0.3296
AC	3.1584	4.6157	25.2669	0.5094
BC	-0.6464	0.9231	-25.8563	0.4997
$\mathbf{A^2}$	-76.3581	17.2238	-305.4324	0.0013
$\mathbf{B^2}$	-2.5714	0.6878	-257.1443	0.0039
$\mathbf{C^2}$	-0.0202	0.6878	-0.3231	0.9772

a. The table for FAME content which obtained from the experiments was analyzed by using software Design-expert 8.0.6.

Table 3-15 The predicted value and experimental productivity under optimum microalgae cultivation condition.

Productivity (g/L/day)	Predicted value	Experimental value		
		1	2	3
Biomass productivity [a]	0.486	0.465	0.427	0.387
Lipid production [b]	0.216	0.195	0.178	0.151
FAME production [c]	0.157	0.163	0.145	0.141

a. The microalgae *Chlorella* sp. TT-1 was cultivated under the microalgae cultivation condition which the initial density of 0.37 g/L aerated with 75 % flue gas ratio at 0.30 vvm aeration rate.

b. The microalgae *Chlorella* sp. TT-1 was cultivated under the microalgae cultivation condition which the initial density of 0.35 g/L aerated with 75 % flue gas ratio at 0.24 vvm aeration rate.

c. The microalgae *Chlorella* sp. TT-1 was cultivated under the microalgae cultivation condition which the initial density of 0.37 g/L aerated with 74 % flue gas ratio at 0.25 vvm aeration rate.

Table 3-16 Biomass and lipid production of *Chlorella* sp. TT-1 cultivated under different illumination. [a]

Illumination ($\mu mol/m^2/s$)	Biomass production (g/L)	Lipid content (%)	Lipid production (g/L) [b]
300	0.846 ± 0.034	39.10 ± 0.28	0.331 ± 0.010
500	1.015 ± 0.037	48.68 ± 0.18	0.494 ± 0.007
700	0.451 ± 0.103	43.25 ± 0.10	0.195 ± 0.029

a. The microalgae *Chlorella* sp. TT-1 was cultivated under the microalgae cultivation condition which the initial density of 0.37 g/L aerated with 75 % flue gas ratio at 0.30 vvm aeration rate. The microalgae in batch culture was incubated for 7 days at 28 ± 2 ℃ and the illuminations of 300, 500, and 700 $\mu mol/m^2/s$ were irradiated in the experiment. *Chlorella* sp. TT-1 at stationary phase was collected after 7 days to measure lipid content.

b. Lipid production (g/L) = (biomass production × lipid content) / 100

c. Each data indicates the mean ± SD from three experiments.

Table 3-17 Lipid composition profile of *Chlorella* sp. TT-1 aerated at different aeration rates. [a]

	Illumination (μmol/m^2/s)		
	300	500	700
Fatty acid composition [b, c]	**Relative content (%)**		
C16:0	38.64 ± 0.94	46.73 ± 3.11	40.64 ± 0.58
C18:0	6.30 ± 0.37	7.32 ± 0.25	6.69 ± 0.17
C18:1	15.68 ± 0.55	13.02 ± 0.08	12.28 ± 0.03
C18:2	18.55 ± 0.23	14.22 ± 1.54	17.09 ± 0.19
C18:3	11.78 ± 1.02	12.23 ± 1.52	15.37 ± 0.18
others	9.05 ± 0.68	6.48 ± 0.52	7.93 ± 0.17

a. The microalgae *Chlorella* sp. TT-1 was cultivated under the microalgae cultivation condition which the initial density of 0.37 g/L aerated with 75 % flue gas ratio at 0.30 vvm aeration rate. The microalgae in batch culture was incubated for 7 days at 28 ± 2 ℃ and the illuminations of 300, 500, and 700 μmol/m^2/s were irradiated in the experiment. Lipid compositions of *Chlorella* sp. TT-1 were analyzed by Gas chromatography.

b. Lipid compositions were analyzed by Gas chromatography.

c. The appropriate lipid composition for FAME are palmitic acid (C16:0), Stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), and α-Linolenic acid (C18:3).