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Primary Factors Affecting Growth of Microalgae Optimal Light Exposure Duration and Frequency

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Primary Factors Affecting Growth of Microalgae Optimal Light Exposure Duration and Frequency

by

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NOMENCLATURE

BBM	Bold’s Basal Medium
TFA	Total Fatty Acid
PBR	Photobioreactor

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ABSTRACT

To cultivate more microalgae and at the same time optimize the land use, the optimal light exposure duration and frequency are important factors in the design a photobioreactor which contained two parts was tested. One part of the PBR was exposed to the light, while another part of the PBR was placed underground without light. Different daily exposure durations (8 hours, 7 hours, 6 hours, and 5 hours) and different exposure frequencies (53 cycles/hour, 70 cycles/hour, 88 cycles/hour, and 105 cycles/hour) were investigated. A comparison of the growth rates and biomass characteristics of microalgae under different light conditions showed that, the biomass concentration in the aqueous phase can reach up to 3.5g/L when the daily light duration was 7 hours and frequency was 70 cycles/hour. This experimental result implies that the concept of this two portions photobioreactor can work to produce microalgae biomass under certain light exposure durations and frequencies. It is a new approach to discover the light-dark cycle for microalgae growing.

CHAPTER I

INTRODUCTION

1.1. Research Description

To save cultivated land area for microalgae, a new photobioreactor (PBR), containing two parts was tested. One part of the PBR was exposed to the light, while another part of the PBR was placed underground without light. To determine that the PBR was appropriate, the optimal light exposure duration and frequency were investigated. The microalgae cell density and cell productivity were measured under different light exposure conditions along with the biomass production contents as presented in Chapter III.

1.2. Thesis Organization

In Chapter II, literature was reviewed to introduce all information of microalgae. Chapter III introduces the materials and methods were used in this research. For example, how the microalgae was cultured and collected, how the PBRs were operated, and how analysis was performed. In Chapter IV, the data shown under each light exposure condition (different light exposure duration and different light exposure frequency), with compared with cell density, cell productivity, cell number counting, protein content and fatty acids content. In Chapter V, general conclusions are drawn and future researches are recommended.

CHAPTER II

LITERATURE REVIEW

2.1. Introduction of Microalgae

Microalgae is a class of plants distributed widely in both terrestrial and marine environments. Currently more than 40 different species of microalgae were studied for multiple purposes such as fresh water environmental protection and biomass production content analysis. Because autotrophic microalgae produces polysaccharides, protein, lipid, it has a promising future in the area of food, medicine, genetic engineering and biodiesel. (Lavens and Sorgeloos, 1996)

Microalgae can be classified into eight classes and 32 genera as shown in Table 2.1, According to the table, the size of microalgae ranges from a few micrometers to more than 100μm. This list contains species of diatoms, flagellated, chlorococcalean green algae, and filamentous blue-green algae. (Laven and Sorgeloos, 1996)

Table 2.1 Major classes and genera of micro-algae cultured in aquaculture (Laven and Sorgeloos, 1996).

Class	Bacillariophyceae	Haptophyceae	Chrysophyceae	Prasinophyceae	Cryptophyceae
Genus	Skeletonema	Isochrysis	Monochrysis (Pavlova)	Tetraselmis (Platymonas)	Chroomonas
	Thalassiosira	Pseudoisochrysis		Pyramimonas	Cryptomonas
	Phaeodactylum	Dicrateria		Micromonas	Rhodomonas
	Chaetoceros				
	Cylindrotheca				
	Bellerochea				
	Actinocyclus				
	Nitzchia				
	Cyclotella				
Class	Cryptophyceae	Xanthophyceae	Chlorophyceae	Cyanophyceae	
Genus	Chlamydomonas Chl.	Olisthodiscus	Carteria	Spirulina	
			Dunaliella		

2.2. Introduction of Biodiesel.

The demand for energy is increasing incredibly, especially in recent decades, while environmental problems are also important issues to be concerned with, so renewable and low pollution energy, such as wind energy, solar energy and geothermal energy must be explored. As a renewable energy, biodiesel is considered a potential alternative diesel fuel globally. For example, in the United States, the production of biodiesel is as high as 691 million gallons in 2008, even though the annual production decreased to as low as 490 million gallons in 2009, the total high production amount is still outstanding (Ethier et al., 2011).

Numerous researches showed that biodiesel has advantages over our current main energy, – petroleum. As a renewable energy source, biodiesel is an environmentally – friendly energy. Biodiesel releases less greenhouse gas, discharges less carbon dioxide and low content sulfur, while also decreasing the discharge of sulfur and carbon monoxide content. Technically speaking, 90% of air toxicity and 95% of cancers can be decreased by biodiesel (Huang et al., 2010). What's more, biodiesel has no need to face the shortage, it can be supplied sustainably. Further, biodiesel has great potential in the energy market since the price of fossil fuel will be incredibly high based on its shortage and the highly increased energy demand, but with biodiesel as a renewable energy source the rising price will not be an issue.

2.3. Introduction of Microalgae Biodiesel

Microalgae biodiesel is one technology in the biodiesel field and a lot of researchers use microalgae to produce chemicals, oils and polysaccharides (Borowitzka, 1922 & Munro

et al., 1999). Although some researches show crops such as corn, soybean and animal fats can produce oil, use as renewable biodiesel after treatment (Ma and Hanna, 1999). These biodiesel cannot meet the increased energy demand since their production is too low, even for the vehicle usage. With photosynthesis, microalgae absorb light to produce oil. However, its efficiency is much better than crops plants, and the capacity of producing oil is also better than the crops plants. (Chisti, 2007). The land usage is also an important issue, comparing to microalgae oil extraction, and large cultivation area needs more crop oil. To meet the demand of 50% of U.S. transportation fuel, the cultivation area of different crops and microalgae are presented in Table 2.2. From the table, if using oil – palm as biodiesel source, 24% of the total cropping area is still under 50% of U.S. transportation fuel demand although it has the highest oil yield level. However, if using microalgae as biodiesel source, the required area of land is as low as 1% - 2.5% of the total cropland to meet the same transport fuel demand. (Chisti, 2007)

Table 2.2 Comparison of some sources of biodiesel. (Chisti, 2007)

Crop	Oil yield (L/ha)	Land area needed (M ha)[a]	Percent of existing US cropping area[a]
Corn	172	1540	846
Soybean	446	594	326
Canola	1190	223	122
Jatropha	1892	140	77
Coconut	2689	99	54
Oil palm	5950	45	24
Microalgae[b]	136,900	2	1.1
Microalgae[c]	58,700	4.5	2.5

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

Microalgae oils have its advantages. For example, the constitution of microalgae is very similar to vegetable oils, and the composition of microalgae is relative single. What's

more, under center operation control, microalgae oil production can be reach to as high as 85% of the dry weigh. Last, less cycle time is needed for microalgae. (Huang et al., 2010) Thus, considering the satiation of increasing energy demand and cultivation land area requirement, microalgae as renewable biodiesel seems to be the most appropriate source to take place of petroleum. Microalgae biodiesel technology is a hot topic which gains numerous attentions recently, a lot of interests on the oil production capability of microalgae to. Microalgae biodiesel has disadvantages such as algal lipids have less fuel value rather than diesel fuel. Also, the production of algal lipids is hard to meet economic challenges because the price for harvesting and dewatering are high, and application for oil extraction is not mature. The photo reactor design for microalgae also needs more experiments.

2.4. Important Component of Microalgae – Lipid and Protein

Generally speaking, under a favorable culture condition, the biomass of microalgae contains protein (30 – 50 %), carbohydrates (20 -40%) and lipids (8 – 15%) (Hu, 2004).

Microalgae diesel technology is using the lipids extract from microalgae biomass. Most species of microalgae have oil levels as 20–50% while some species oil content in microalgae (such as *Schizochytrium* sp.) can exceed 80% of dry biomass weight. Oil productivity is defined as the mass of oil produced by unit volume of the microalgae at one day, which relates to the oil content of the biomass and the algal growth rate (Chen, 2011). High oil productive microalgae are good for biodiesel production. It is necessary to have an ability to economically produce large amount of oil-rich microalgae biomass to produce algal oils. (Chisti, 2007)

Protein content various based on different microalgae species, from 15-71% of dry weight. According to recent researches, algal protein is considered as an optimal source for animal feed since algal protein has been estimated that has a considerable profile of amino acid (Gross, 2013). In 2007, 30% of the global algal was cultured for animal feed because of the protein content of algae. (Gross, 2013 & Becker, 2007)

2.5. Photosynthetic

The photosynthetic is a progress for green plant using light energy to convert carbon dioxide and water to organic compound (typically glucose), while releases oxygen gas. The basic chemical equation can be summarized as below (Carvalho et al., 2011):

$$6H_2O + 6CO_2 \rightarrow C_6H_{12}O_6 + 6O_2$$

There are two phases of photosynthetic, the first stage is photoreaction. During this stage, the chlorophyll in green plants can produce electronic in order to convert the light energy of sunlight into electrical energy. The electrons will be transported though thylakoid membrane, while transfer the H^+ proton from chloroplast stroma to thylakoid lumen, to build electrochemical proton gradient for ATP synthesis. The last step of photoreaction is electrons accepted by $NADP^+$, it will be reduced to NADPH. In photoreaction, water can be decomposed into oxygen and hydrogen, oxygen is released out of absorbed by the chlorophyll molecules is also further converted to chemical energy, and these chemical energy can be stored in the adenosine triphosphate. The chemical equation of photoreaction can be summarized as below:

$$2H_2O \rightarrow 4\,[H] + O_2 \uparrow$$

$$ADP + Pi \rightarrow ATP$$

The second stage of photosynthetic is dark reaction, it is a cycle that continuous consumption of ATP and NADPH and CO_2 fixation reaction while produce glucose, also known as the “Calvin cycle”. Because of Calvin observed the process how CO_2 converted into organic using “C” labeled CO_2. In the dark reaction phase, hydrogen reduction cannot directly reduce the carbon dioxide which is absorbed through the stoma by the green plants from the air. It must first combine with the C_5 (a five-carbon compound, ribulose diphosphate) in the plant, this process is called the fixation of carbon dioxide. After a carbon dioxide molecule is fixed by a C_5 molecule, two C_3 (a three-carbon compound, 12 glyceraldehyde 3 - phosphate) molecule will be formed. With enzyme catalysis, C_3 molecule will receive energy which is released by ATP hydrogen reduction. Subsequently, a number of reduced C_3 will form carbohydrate; the rest C_3 will change back to C_5 again, so that the dark reaction stage preceded continuously, named carbon-fixation reaction. Carbon fixation reaction began in the chloroplast stoma, finished in the cytoplasmic matrix. In this dark stage, the reactions ATP and NADPH which are produced in photoreaction as energy, and also fix CO_2 to transform them into glucose , the process does not require light that the reason why it is called dark reaction. The chemical equation of dark reaction can be summarized as below:

$$CO_2 + C_5 \rightarrow 2C_3$$

$$C_3 + [H] \rightarrow (CH_{2O}) + C_5$$

The dark reaction time will be influenced with shortage of light, because of the lack

of ATP which generated in photoreaction. But not the carbon procedure will not stop immediately when the light disappear because the residual ATP part will continue to provide the conditions for the carbon reaction. Carbon reaction will stop if the situation of light shortage period is a long time (Carvalho et al., 2011).

According to the discussion above and back to microalgae growth, long dark reaction period will lead biomass loss, while the growth rate of microalgae decreasing because the respiration processes of microalgae will produce more carbon dioxide. The growth of microalgae will be considerable if the light/dark cycle can be controlled well. Several lab researches have been worked on the light/dark cycle. (Carvalho et al., 2011)

2.6. Microalgae Growth Factor

2.6.1. Nitrogen/phosphorus nutrient

Nitrogen is an essential element for growth of microalgae. Nitrogen has a wide source; some species of microalgae can fix the nitrogen gas in the air though nitrogen fixation process for their own use. The increased amount of nitrogen content in growing conditions will increase the growth of microalgae. The form of nitrogen also can affect growth of microalgae: NH_4– N, NH_3– N and NH can also be useful nutrient element for microalgae, according to research, the NH_4– N is easier for microalgae to absorb than NH_3– N. (Dortch, 1990 & McCarthy and Wynne, 1982)

Optimal phosphorus concentration is conducive to growth of microalgae. When TP ≤ 0.045mg/L, the microalgae growth will be prohibited. High concentration of phosphorus TP ≥ 1.65mg/L also cannot significantly promote microalgae growth rate (Dortch, 1990). When TP equals to 0.02mg/L, microalgae can grow well, but the concentration of phosphorus has no promotion to growth rate of algae when TP ≥ 0.2mg/L (Xu et al., 2006). Microalgae also

select different formation of phosphorus, using different phosphors source to study, the result of research showed that dipotassium hydrogen phosphate is the biggest consumption for microalgae, which means this form of phosphorus is useful for microalgae growing (Wu et al., 2012).

In aquatic ecosystems, in addition to nitrogen and phosphorus element, the ratio to nitrogen and phosphorus is also used as a key factor to test the density of microalgae cell density (Tilman, 1982, Hosub, 2007). The nitrogen and phosphorus ratio (N / P) directly affect the growth of microalgae, composition of cells and nutrient uptake (Anderson, 2003). According to Redfield's law, the atomic ratio C: N: P in algae cells is 106:16:1, when the N/P ratio exceeds 16, concentration of phosphorus will be considered as a limited factor. If less than 16, nitrogen content needs to be controlled to make sure optimal growing condition for microalgae (Redfield et al., 1963). However, different species of microalgae has different atomic ratio in cells, the requirement for nitrogen and phosphorus will be various (Sun et al., 2006).

2.6.2. Character of light

Light is an essential key for growth of microalgae. Microalgae uses light to process the photosynthetic, but the light energy cannot be stored by microalgae, so the light should be supplied sustainably. The microalgae cannot use all the supplied light because microalgae cannot absorb all the photons, and too much light will cause light inhibition for the surface layer of microalgae. The inner portion microalgae cannot reach the light and lack of photons.

Below is the electromagnetic radiation spectrum of light, as showed in Figure2.1. Through the photosynthetic process, for autotrophic microalgae to convert carbon dioxide in the air into organic compounds, visible light is the main source of energy (Carvalho et al.,

2011) since the chlorophylls, phycobilins and carotenoids in microalgae can be absorbed in the visible light range (Table 2.3).

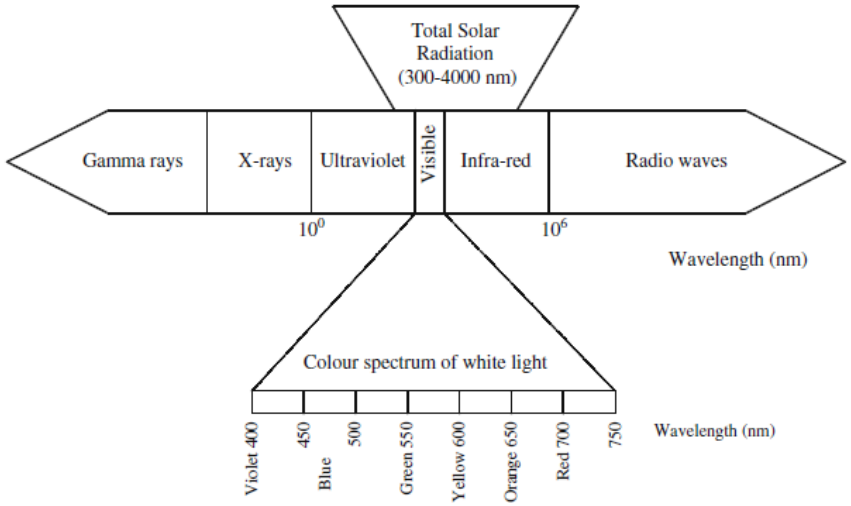

Figure 2.1. Whole electromagnetic spectrum with detailed spectral pattern of visible light. (Carvalho et al., 2011)

Table 2.3 Photonic features of major pigments in microalgae. (Carvalho et al., 2011)

Pigment group	Color	Ranges of absorption bands (nm)		Pigments
Chlorophylls	Green	450–475	Hydrophobic	Chlorophyll *a*
		630–675		Chlorophyll *b*
				Chlorophyll c_1, c_2, *d*
Phycobilins	Blue, red	500–650	Hydrophilic	Phycocyanin
				Phycoerythrin
				Allophycocyanin
Carotenoids	Yellow, orange	400−550	Hydrophobic	β-Carotene
				α-Carotene
				Lutein
				Violaxanthin
				Fucoxanthin

Light saturation is defined by a saturation constant of light (Figure 2.2), which is the intensity of light where the specific biomass growth rate is 50% of its pick value, μmax. Light saturation constants for growth rate of microalgae tend to be less than the maximum sunlight intensity level which happens in the middle of the day.

When the light intensity above a certain value, continue increasing in light intensity level will decrease the microalgae growth rate actually (Figure 2.2.). This is called photoinhibition phenomenon. Microalgae become photoinhibited when light intensities a little bit higher than the light intensity at the specific growth rate peaks. Because of excessive light source, photoinhibition phenomenon will cause generally reversible damage to the photosynthetic process (Rubio et al., 2003). Avoiding photoinhibition can help to increase the daily growth rate of microalgae biomass. (Chisti, 2007)

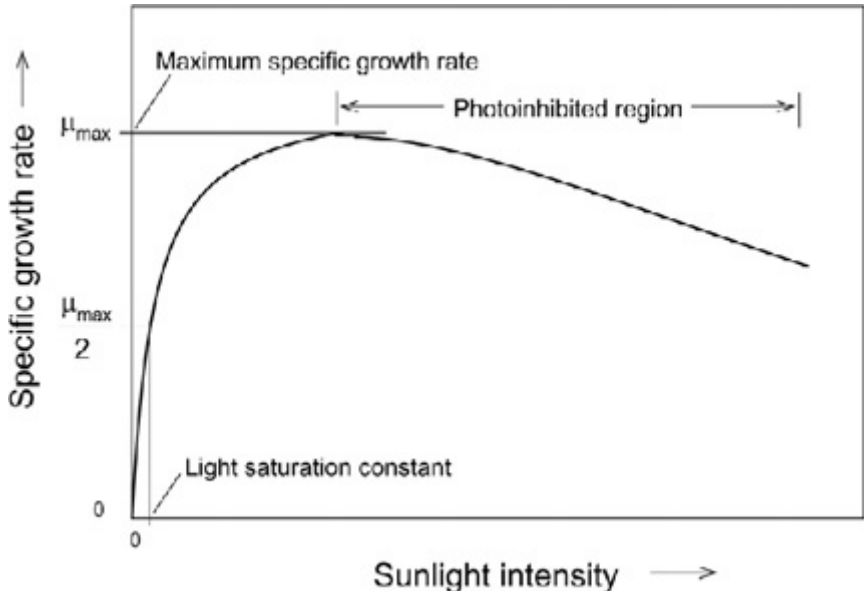

Figure 2.2. Effect of light intensity on specific growth rate of microalgae. (Chisti, 2007)

2.6.3. Temperature

With the light intensity changing, temperature is an environmental factor which indirectly affects growth of microalgae (Huang et al., 2008). According to Takemura's study on the effects of temperature on the maximum rate of photosynthesis of microalgae in Lake Kasumigaura, the results shows that when the temperature of water lower than 4 $^{\circ}$C the photosynthesis of microalgae was completely inhibited. When the temperature is between 4

°C to 11 °C, photosynthesis is substantially inhibited. After temperature is higher than 11 °C, the relationship between temperature and growth of microalgae is linear (Takemura et al., 1985). Temperature determines the activity and reaction rates of intracellular enzyme, which will have an influence on algal photosynthesis, respiration intensity, affect the growth of microalgae and to limit its distribution (Tan et al., 2009).

2.6.4. pH and salinity

Water pH related to growth of microalgae tightly. Through photosynthetic, the pH will be changed, which is already discussed in photosynthetic part. The pH value will also affect the growth rate of microalgae, it will be easier for microalgae to capture CO_2 in the atmosphere when the growing condition is alkaline, which can produce more biomass (Zang et al., 2011 & Melack, 1981). With the increase of pH, CO_2 into water transferred into HCO_3^- which is the mainly existing formation of carbon in weak alkaline. And this also can be used by microalgae majorly. But according to Liu's study, the content of chlorophyll of microalgae will decrease when the pH value goes from 8.5 to 9.5 (Liu et al., 2005).

Microalgae has its own system to adjust salinity range. Generally, seawater microalgae can tolerate higher salinity rather than fresh water microalgae (Zhu et al., 2003). Studies showed that microalgae has its own optimal growth salinity, when salinity higher or lower than this will be harmful to algal growing rate. For example, when in the low salinity growing condition, it will be helpful for algal growth with the addition of NaCl and $NaSO_4$ but when the salinity higher than 6g/L, the growth rate of microalgae will be prohibited (Liu et al., 2006).

2.6.5. Mixing

Shading is a problem in growth of microalgae, it will prohibit microalgae to absorb light effectively, and it will affect biomass production. In general, microalgae grow well in lake or stream because of the dynamics of water (Wang, 2006). When design the PBRs for microalgae, dynamic is also important. Gas mixing can be treat as water dynamic for growth of microalgae. Gas mixing in the PBRs can promote every microalgae cell to obtain equal light source and nutrient.

2.7. Microalgae Cultivation

2.7.1. Open ponds

As an open pond for microalgae growth, it can be characterized into natural sources such as lakes. Shallow big ponds, tanks, circular ponds and Raceway Ponds are the most commonly used systems (Demirbas, 2010). Take the raceway ponds for example, the application of raceway ponds for microalgae biomass culture was used as early as 1950s. The depth of a raceway pond is about 0.3 m, which is made of a closed loop recirculation channel. As shown in Figure 2.3. As the arrows show, flow is moved around bends in the channel by baffles placed. The raceway channels are made of concrete or compacted earth along with the plastic. The culture is fed in front of the paddlewheel at the daytime, and the broth is behind the paddlewheel (Chisti, 2007). According to Sheehan's study, the production of microalgae biomass for producing biodiesel is evaluated that the Raceway ponds cost less money than PBRs, which is introduced below. However, the productivity of biomass is also lower than PBRs. (Sheehan etal. 1998.)

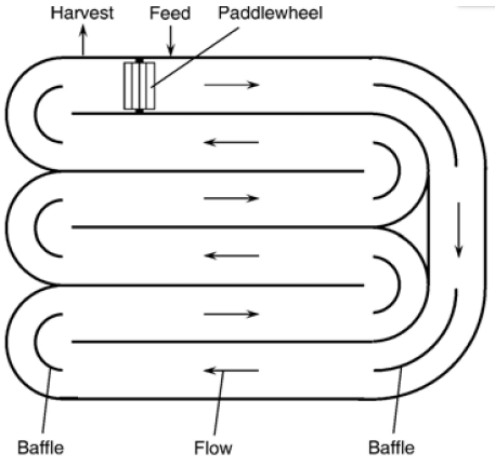

Figure 2.3. Arial view of a raceway pond (Chisti, 2007)

2.7.2. Photobioreactors

The applications of PBRs for producing large amount of microalgae biomass are successful. (Molina et al., 2000) Compare to open ponds, PBRs is more expensive but produces more biomass, single-species culture of microalgae is allowed by PBRs for prolonged durations (Chisti, 2007). Under certain good control of PBRs, the production of long chain fatty acids and high value such as DHA and EPA is highly recommended. (Huang et al., 2010) PBRs can be characterized as tubular PBRs, flat-plate PBRs, internally illuminated PBRs and vertical-column PBRs based on the shape and working theory.

2.7.2.1. Tubular PBRs

Tubular PBRs, as shown in Figure 2.4, is designed for mass cultures for outdoor use. It has large illumination surface area, and relatively low cost. However, it requires large space to set up and the temperature control for tubular PBRs is not well established. Additionally, it can increase the PH of the culture, which can cause re-carbonation of the cultures and as a result, raise the price for the reactor. (Ugwu et al., 2008)

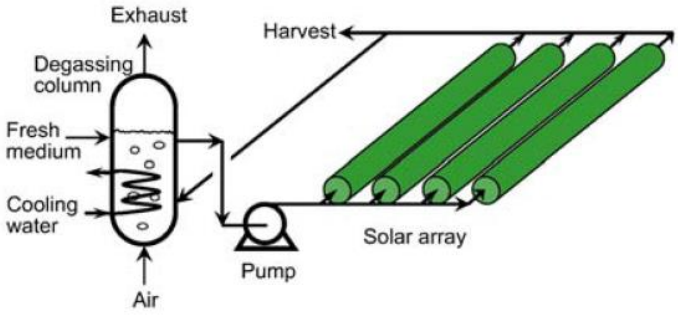

Figure 2.4. A tubular PBR with parallel run horizontal tubes. (Ogbonna et al., 1999)

2.7.2.2. Flat-plate PBRs

Flat-plate PBRs, as the name shows, has large surface area for illumination. It always made of transparent materials in order to maximize of receiving solar light. It is good for outdoor culture and immobilization of algae, relatively low cost and easy to maintain. However, the temperature control is still an issue. What's more, there is a possibility to hydrodynamic stress to some algal strains. (Ugwu et al., 2008)

2.7.2.3. Internally-illuminated PBRs

As shown in Figure 2.5., the Internally-illuminated PBRs can be illuminated internally with fluorescent lamps. The research (Ogbonna et al., 1999) shows that it can utilize energy of both artificial and solar light, which means the artificial light can be used as back up source to improve the light intensity continuously, both daytime and nighttime.

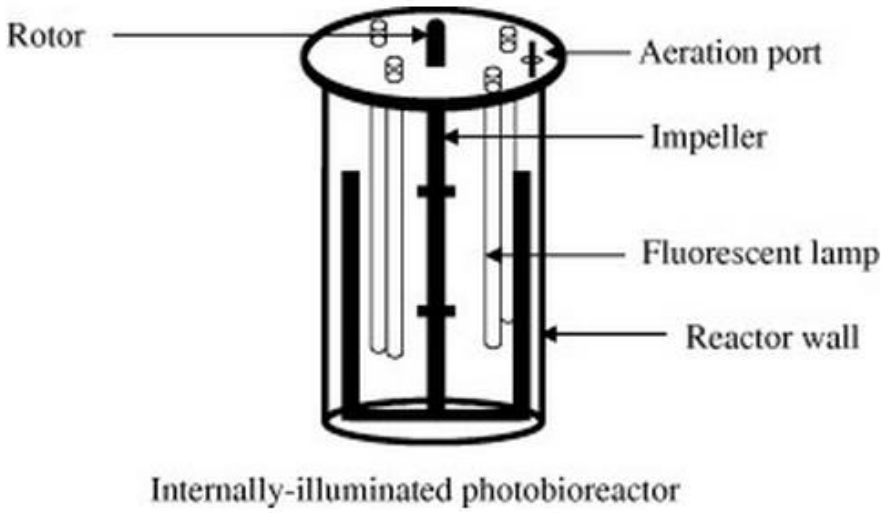

Figure 2.5. Schematic diagram of an internally illuminated PBRs. (Ogbonna et al., 1999)

2.7.2.4. Vertical-column PBRs

Vertical-column PBRs are usually small in size, relatively low cost and user friendly (Sánchez et al., 2002). The advantages of vertical-column PBRs can be summarized as good mix ability with low energy, high mass transfer, and good temperature control and photo-inhibition &photo-oxidation reduction. However, Vertical-column PBRs have smaller surface area for illumination and some sophisticated materials are needed for construction.

2.8. Light / Dark Cycle for Microalgae

It is no doubt that the light factor plays an important role in photosynthetic progress of microalgae. In order to produce high level of microalgae biomass, the efficiency of PBRs and illumination is required. Using light and dark cycle pattern for illumination design has been studied for a long time (Kok, 1953). So far, the researchers focused on changing light intensity, using short alternated light dark time as light source for cultivation of microalgae, such as flashing light. Attitudes on flashing light are summarized as follows. First, when using flashing light for microalgae's photosynthesis, the efficiency is never higher than using

steady light as light resource which with same intensity or with equal average light intensity Second, with same light intensity for both flashing light and steady light, the capability of microalgae to absorb the flashing light is higher than when microalgae utilized continues illumination. Third, when the incident intensity is high enough, there is no need to require a strict flash time to achieve an outstanding efficiency boost. Fourth, flashing light is approved to have some other advantages over steady light resource, powder consumption will be reduced on according to existence of off-cycle. Because of lower heat generation, cooling system is not needed which will save a big amount of costs and also make the process being easier; instantaneous photosynthetic photo flux will be increased. However, during lab experiments, it is hard to calculate the flash light intensities since the flashing light intensity changes quickly timely, and there is a long dark period existing comparatively, which may interrupt the quantum sensor from averaging the light over a cycle. Therefore, more detailed researches should be studied on maximum and average light intensities, duty cycles and frequency also need to be tested before accomplish the advantage of using flashing light on growth of microalgae. (Kim et al., 2005)

Finding critical cell density of microalgae is a profitable engineering tool for flashing light in algal application. The critical cell density of microalgae can be calculated using cell concentration, average cell volume, and the surface/volume ratio of a culture reactor. Based on estimating critical microalgae cell density and specific oxygen production rate, the effectiveness of flashing light for photosynthetic efficiency can be tested. With increased instantaneous photosynthetic photo flux, the specific oxygen production rate was enhanced under flashing light. Under different frequencies and duty cycles, the efficiency of photosynthetic per unit volume was relatively constant, which is around 0.8 mol $O_2\,\mu m^{-3}h^{-1}$

Thus, the flashing light frequency and duty cycle don't have an obvious improvement on the rate of specific oxygen production. Meanwhile, the flashing frequency will influence on the specific oxygen production rate a little amount when the frequency is 10 - 50 kHz. (Park and Lee, 2001)

2.9. Microalgae Seed – *Scenedesmus dimorphus.*

Scenedesmus dimorphus is a freshwater unicellular microalgae, which belongs to Chlorophyceae class. It is a strain of microalgae that can grow rapidly and synthesize considerable amount of desirable product such as protein and lipid. The specific growth rates have been reported in the range of 0.8 day^{-1} to 1.6 day^{-1} (Welter et al., 2013). The biomass *of S. dimorphus* contains 35% protein, 60% carbohydrate or 37% total lipid (Wang et al., 2013). *S. dimorphus* is tolerant to wide range pH from 6.5 to 8, and also has a great capability of tolerance to high gas concentration of carbon dioxide and nitric oxide It can tolerant the sulfur dioxide concentration as much as 100 ppm. What's more, the *S. dimorphus* is robust to mental contamination such as copper (Jiang etal., 2013 & Nalewajko et al., 1997).

In tropical countries such as Colombia, in order to solve the problem of lack of water for crops, researches are interested on reuse the treated wastewater, which is using *S. dimorphus* to remove ammonia and phosphorus in agroindustrial wastewater. The results showed the removal efficiency on ammonia was as high as 95% (Gonzalez et al., 1997), while the removal efficiency on phosphorus is 57% (Proulx et al., 1994). *S. dimorphus* presents as a good alternative to treat agroindustrial wastewater, compared to those obtained in other studies with *cyanpbacteria* like *Phormidium* and *Sprirulina*. (Gonzalez et al., 1997)

In Kang's study, the concept of producing *S.dimorphus* biomass using waste ammonia gas from animal house shows a promising result. The microalgae not only can remove ammonia gas, but produce biomass as animal feed as well. She also pointed out more experiments need to be done since the growth condition for microalgae can be different when close to animal house situation in real. Such as the carbon source for microalgae can also from CO_2 and CH_4 that concentration will be high in animal house. (Kang, 2012)

CHAPTER III

MATERIALS AND METHODS

3.1. Cell Strain and Substrate

The microalga *Scenedesmus Dimorphus* (UTEX 1237) was used. The microalgae was obtained from the culture collection at University of Texas at Austin. The seed of *S.dimorphus* was maintained in 250-ml autoclaved Erlenmeyer flasks. To culture the seed of *S. dimorphus*, 5mL of old *S. dimorphus* seed was weekly transferred to a 250-mL autoclaved Erlenmeyer flask which contained 50mL Bold's Basal Medium (BBM) (Bold, 1949 & Bold and Bischoff, 1963), and was marked as new generation of *S.D.* seed. All procedures were done in sterile environment. The flasks were placed at 25^{o}C on an orbital shaker setting at 200 rpm, the light intensity of continuous illumination was set to 110-120 µmol s^{-1} m^{-2}. The BBM contained KH_2PO_4 (17.5 g/L), $CaCl_2 \cdot 2H_2O$ (2.5 g/L), $MgSO_4 \cdot 7H_2O$ (7.5 g/L), $NaNO_3$ (25 g/L), K_2HPO_4 (7.5 g/L), NaCl (2.5 mg/L), EDTA (50 g/L), KOH (31 g/L), $FeSO_4 \cdot 7H_2O$ (4.98 g/L), H_2SO_4 (1mL), H_3BO_3 (11.42 g/L), MoO_3 (0.71 g/L), and trace metal solution (1ml/L) which includes $ZnSO_4 \cdot 7H_2O$ (8.82 g/L), $MnCl_2 \cdot 4H_2O$ (1.44 g/L), $CuSO_4 \cdot 5H_2O$ (1.57 g/L), $Co(NO_3)_2 \cdot 6H_2O$ (0.49 g/L). The BBM was autoclaved at 270^{o}F for one hour.

3.2. Photobioreactors Setup

The microalgae was exposed to different light exposure patterns, the PBRs were glass columns (25.4 mm in diameter, 61 cm in length), and working volume was 600 mL. Each glass column was paired with same reservoir but different working volume (Figure 3.1.). Each set of reactor set included a glass column, a dark medium reservoir, and a recycle pump.

The reservoirs were of medium size (0.83 L - 2.284 L in this study); with different active volumes set and was completely covered with aluminum foil to avoid exposure to sunlight. Two sets of reactor were tested at one time. One lab set was for mainly observation, which another was prepared as same as first one to do the repeated lab. Medium was continuously re-circulated by the recycle pump from the reservoir to the column. The size of the reservoir controled the exposure duration while the pump rate controled light exposure frequency. As shown in Figure 3.1, circular bubble tube was fixed on the bottom of the reservoir to make sure microalgae not attached on the wall of reservoirs. Additionally, bubble air in the inlet of the pump was allowed to prevent microalgae to attach on the wall of photo - reactor columns. Brush both reservoirs and photo – reactor column after each lab to make sure there were no remain microalgae cell.

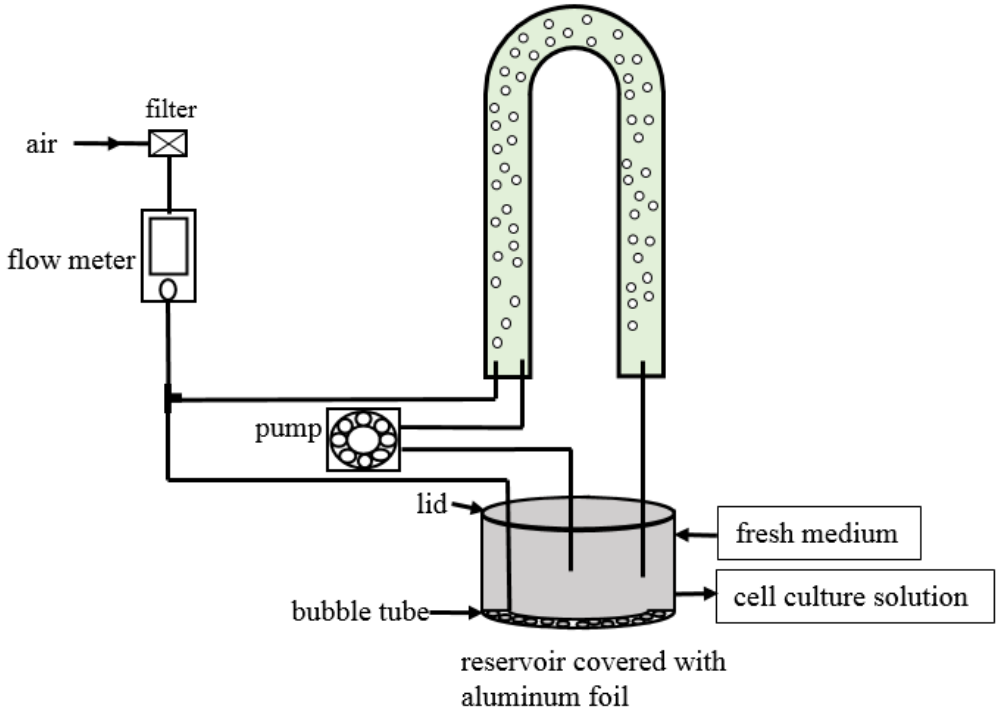

Figure 3.1. Schematics of the photobioreactors system for microalgae growing.

3.3. Photobioreactors Operation

The columns were illuminated by fluorescent lamps (Ecoiux F32T8.SPX50-ECO) with an intensity of about 150 µmol $m^{-2}s^{-1}$. The culture was mixed at aeration rate of 0.25 vvm (volume gas / volume liquid / min) (Jiang et al., 213). The flow meter (Cole – Parmer: PERI – 010266. Chicago, IL) was used in this study to adjust the gas flow rate, while pH was also maintained daily in the certain range of 6.95-7.00 by a pH meter (Jenco, 6230N). A room temperate of 25^{o}C was maintained during the entire experimental period.

3.3.1. Continuous Culture

The PBRs was operated in a continuous mode. Since the optimal dilution rate is 0.1 (Kang, 2012), 10% cell suspension of total sample volume from each reservoir were removed daily for cell density tests, and same amount of BBM were added into each reservoir. The steady state can be observed after 18 days, at this state sample were collected for protein, fatty acid, cell number counting and element content analysis.

3.4. Analysis

3.4.1. Cell Growth Analysis

Optical density of microalgae biomass was measured by spectrophotometer (DU 720, Beckman Coulter, Fullerton, CA) at 680 nm (OD_{680}). The sample was diluted in the range from 0.1 to 1 to observe the optical density. After getting biomass dry weight, the relationship between biomass concentration and optical density can be written as the following equation: Y=1.0319X-0.0346 (R^2 = 0.9952), where Y indicates the biomass

concentration (g/L) and X indicates the optical density (OD_{680}). Optical density values with the dilution factor of 9 were recorded daily, the cell concentration can be calculated from the equation, and the cell productivity (g/L•day) can be calculated by the biomass concentration multiply the dilution rate 0.1 (Kang, 2012).

To make sure whether microalgae was growing to steady status, counting cell number of microalgae will be done following Hemocytometer method. According to optical density observation, after the microalgae went to steady state, dissolved 10mg freeze-dried sample into 5ml distill water, and the density of samples will be 2 g/L.

3.4.2. Biomass Analysis

When microalgae grow into steady state, sample will be collected in the following five days for biomass analysis. All collected sample were freeze-dried, for fatty acids analysis, protein content analysis, and elements (C, H, N, and S) analysis. Fatty acid analysis was determined by using the previous protocols (Pyle et al 2008) Protein content was calculated as nitrogen percentage × 6.25. Use elemental analyzer to analysis the CHN content of biomass, the element analysis was performed in center for sustainable environmental technologies.

3.4.3 Statistic Analysis

One-way ANOVA was used in this study to test if different light condition will change the cell density and cell productivity of *S. dimorphus* or not. JMP Pro10 was used as the statistical analysis tool. The α level was set as 95%, while the *p* – values of 0.05 will indicate if the differences will be significant or not. In this research, *S.dimorphus* samples

were taken every day when the growth rate went to steady state. Duplicate samples for both cell growth analysis and biomass analysis.

CHAPTER IV

RESULTS AND DISCUSSION

4.1. Cell Growth Rate in Whole Research

Light plays an important role in growth rate of microalgae, as discussed in Chapter II. Under different light exposures, durations and exposure frequencies, the growth rate of microalgae can vary. Figure 4.1 shows the cell density of the microalgae in the entire continuous culture under different growing conditions. In the first stage of this research, exposure duration was determined by changing the medium volume of the equipment. The equation of daily light duration calculation can be written as follows:

$$\text{Daily light exposure duration} = \frac{\text{Volume under light}}{\text{Total Volume}} \qquad \text{Equation 4.1.}$$

Table 4.1 shows the calculations of exposure duration and relative exposure frequency when randomly choosing the same pumping rate of 40 ml/sec. After confirming optimal exposure duration, exposure frequency was determined under the same light exposure duration per day by changing the pumping rate. The research started with durations of 10 hours/day, 8 hours/day, and 6 hours/day. The reason for starting with 10 hours/day is because normal daylight is around 10 hours/day, and the lab-scale project is a pre-test for outdoor scale. When the results showed the cell density has an optimal value at 6 hours/day, 7 hours/day and 5 hours/day, these two durations were chosen to compare the cell density of microalgae under 6 hours/day exposure duration. When the optimal duration equals 7

hour/day and the frequency is 70 cycles/hour, based on the changing pumping rate, the equation of light exposure frequency calculation can be written as follows:

$$\text{Light exposure frequency} = \frac{3600}{\text{total volume / pumping rate}}$$ Equation 4.2.

This research tested exposure frequency starting around 70 cycles/hour, and 53 cycles/hour 105 cycles/hour and 88 cycles/hour were chosen to compare with the cell density when the frequency is 70 cycles/hour. Table 4.2 shows the calculations of exposure frequency with different pumping rates, but same exposure duration of 7 hours/day.

Table 4.1 Total daily exposure duration (hours) when pumping rate is 40 ml/sec.

Reservoir volume, L	Total daily exposure, hr.	Light frequency, cycles/hr.
0.83	10	101
1.2	8	80
1.455	7	70
1.8	6	60
2.284	5	50

Table 4.2 Light exposure frequency when duration is 7 hours/day.

Pumping rate, mL/sec	Light frequency, cycles/hr.
30	53
40	70
50	88
60	105

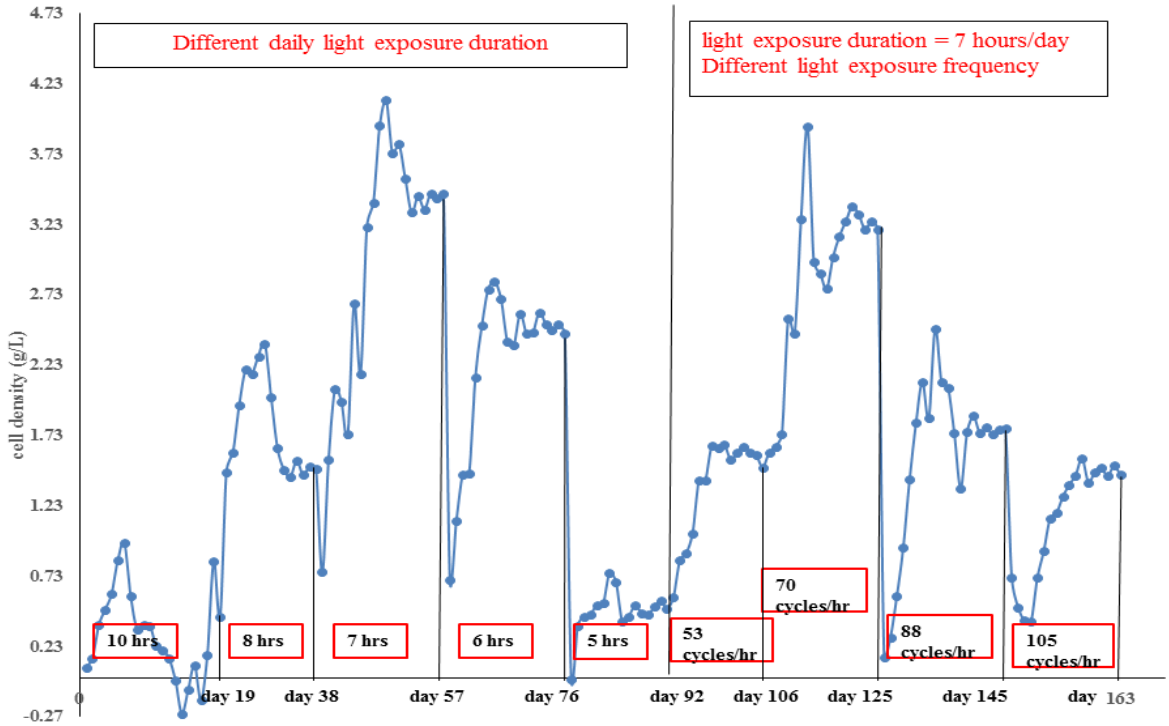

Figure 4.1. Cell density of *S. dimorphus* under different exposure condition includes both duration and frequency tests in continuous culture. The dilution rate is 0.1 day^{-1} in the entire test.

According to Figure 4.1 above, when light exposure equals 10 hours/day, steady state is not unstable and the cell density is too low to collect sufficient samples for further analysis, so in the further analysis, samples under this condition were not analyzed more. The remaining conditions show a full analysis.

4.2 Cell Growth at Different Light Exposure Duration

Exposure duration is a way to find how long the microalgae need the light exposure in order to proceed to photosynthesis. Less light exposure duration will affect the growth rate of microalgae, as the light is not enough for microalgae to absorb, while too much light exposure will cause light prohibition in microalgae. This research uses the autotrophic

microalgae species *S. dimorphus* to test optimal exposure duration first, after determining the exposure duration, then tests the exposure frequency next, based on the optimal exposure duration.

Cell density was calculated every day though the optical density of the microalgae sample. As shown in Figure 4.1 above, the steady state of each growing condition occurs after 10 days. During each steady state, samples are collected for elemental, protein, and fatty acids analysis.

Figure 4.2 below shows the cell density and cell productivity of the microalgae *S. dimorphus* under different light exposure durations, from which the cell productivity can be calculated as (Kang, 2012):

$$\text{Biomass productivity} = \text{cell density} \times D \qquad \text{Equation 4.3.}$$

Where D indicates the dilution rate (day^{-1}) which is 0.1 day^{-1} (Kang 2012).

Since the dilution rate (0.1 day^{-1}) is used constantly in this entire study, the columns of cell density and cell productivity have same area, even though the axis is different. Under durations of 5 hours/day and 6 hours/day, the dark reaction of photosynthesis will be influenced by the shortage of light, because of the ATP which is generated in photoreaction. From 5 hours/day to 7 hours/day, the cell density increases from 0.47 g/L to 3.42 g/L, while the cell productivity increases from 0.047 g/L to 0.34 g/L. This occurs because when the light supply goes up, the photosynthetic efficiency goes up, and the microalgae can use enough light to produce more biomass; hence, this period can be called “light saturation constant” (see Chapter II). When the microalgae gets one more hour of exposure duration, at 8

hours/day, the cell density drops to 1.38 g/L and cell productivity goes down to 0.138 g/L, since the photo-inhibition will occur in the second stage of photosynthesis.

Cell density also can be determined by counting microalgae cell numbers. Figure 4.3 shows the cell number counting results when the *S. dimorphus* reaches steady state under different exposure durations. When the duration varies from 5 hours/day to 7 hours/day, cell density goes from 1.4×10^8 cells /mL to 1.75×10^8 cells/mL, and when the duration is raised to 8 hours/day, the cell density drops to 1.0×10^8 cells/mL. This test of cell density shows the same trend as the one depicted in Figure 4.1. Thus, the shortage of a light source will decrease the cell density of microalgae, while excessive light will cause photoinhibition. The test is done after all conditions are performed: dilute the freeze-dried sample into distilled water, so those counting numbers include both live and dead microalgae cell numbers.

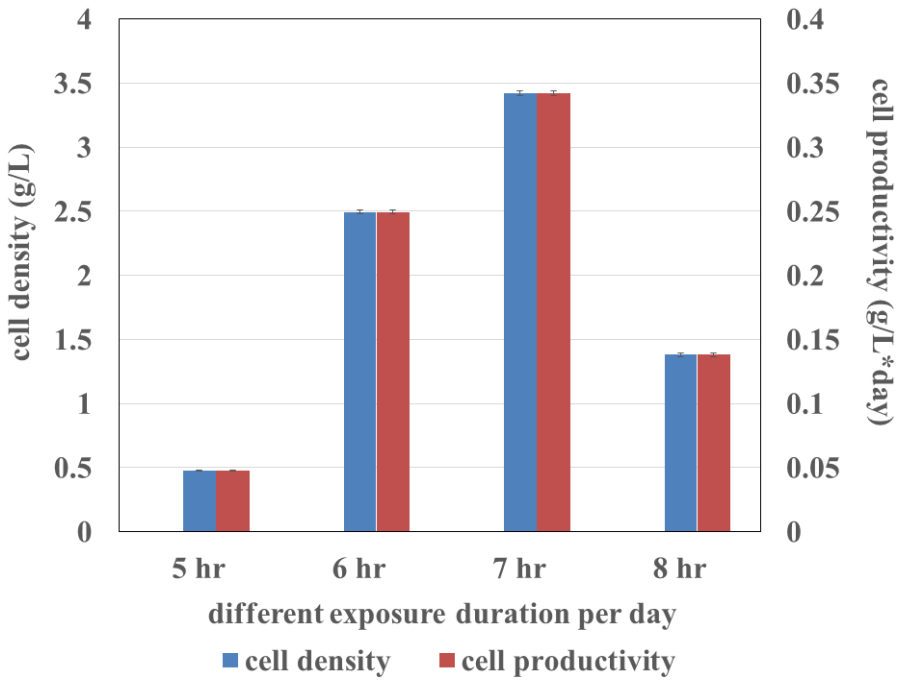

Figure 4.2. Cell density and cell productivity under different exposure duration, when expousre duration equals to 5, 6, 7 and 8 hrs.

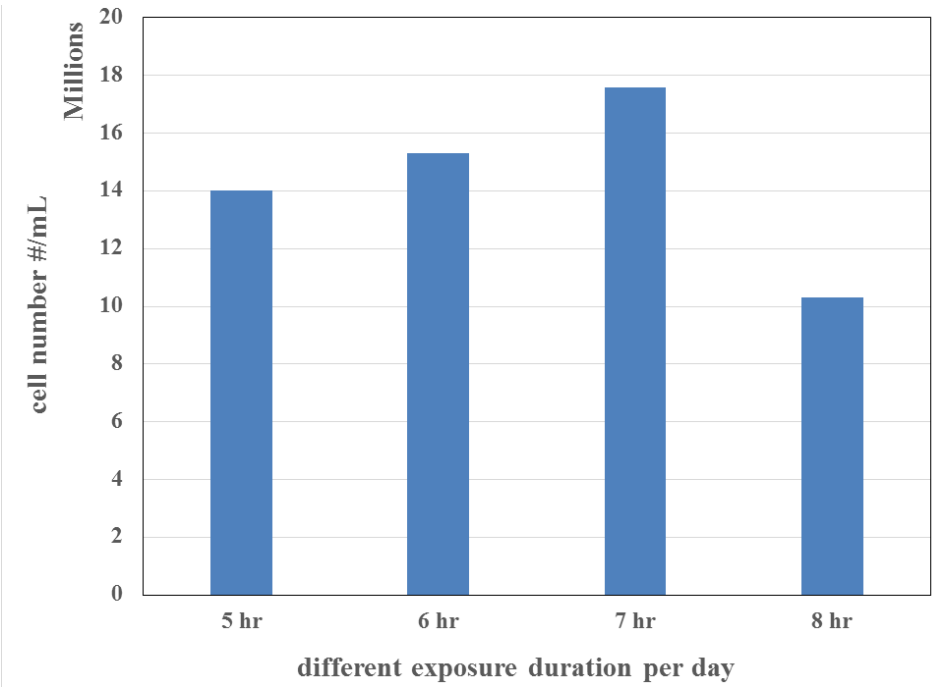

Figure 4.3. Microalgae cell number counting underdifferent exposure duration, when exposure duration equals to 5, 6, 7 and 8 hrs.

4.2.1 Biomass Character at Different Light Exposure Duration

4.2.1.1 Protein and Fatty Acid Analysis

Proteins and fatty acids are two important contents in algal biomass. Different species of microalgae contain varying percentages of proteins and fatty acids. More efficient photosynthesis will produce more proteins and fatty acids. Further, protein-rich biomass can be used for both animal feed and human consumption (Singh, 2011). Acoording to Kang's research, the biomass of *S.dimorphus* offers promising as animal feed, since it has relatively balanced amounts of some kinds of protein, such as methionine, isoleucine, and histidine with lysine, but more experiments needed to be performed since compare to ideal protein contents in animal feed, *S.dimorphus* biomass contains higher amounts of threonine, leucine, valine, and arginine, and lower amounts of cysteine and tryptophan. Figure 4.6 below shows the protein and fatty acid percentages of *S. dimorphus*. The protein percentages under all

duration conditions are in the range of 25% - 30%, lower than 35%, which was mentioned in Chapter II, but according to Bruton et al. (2009), the protein percentage is 8% - 18% of dry matter basis for *S.dimorphus*. Comparing different duration conditions, even the slope of trend is small: the trend of protein percentage in Figure 4.6 goes up from 25% to 30% first, then drops to 24.5% . The protein percentage under 7 hours of exposure duration daily shows a slightly higher result than under other duration conditions.

However, the percentage of fatty acids in *S.dimorphus* under different exposure durations show little difference; in fact, the percentage of fatty acids are relatively similar in this research. The fatty acid composition of *S. dimorphus* under different exposure durations is presented in Table 4.3 below. The algae had a relatively simple fatty acid profile, with palmitic acid (C16:0), linoleic acid (C18:2) and alpha-linolenic acid (C18:3) being the major fatty acids, and pentadecanoic acid (C15:0), oleic acid (C18:1 cis-9), elaidic acid (C18:1 trans-9) and arachidic acid (C20:0) being the minor fatty acids. The percentage of elaidic acid (C18:1 trans-9), however, at under 7 hours/day duration, is significant higher than other duration conditions. The remaining percentages of each individual fatty acid (% TFA, total fatty acid) are relatively stable. Thus, with under 7 hours' exposure duration per day, microalgae will produce more elaidic acid (C18:1 trans-9) rather than other conditions.

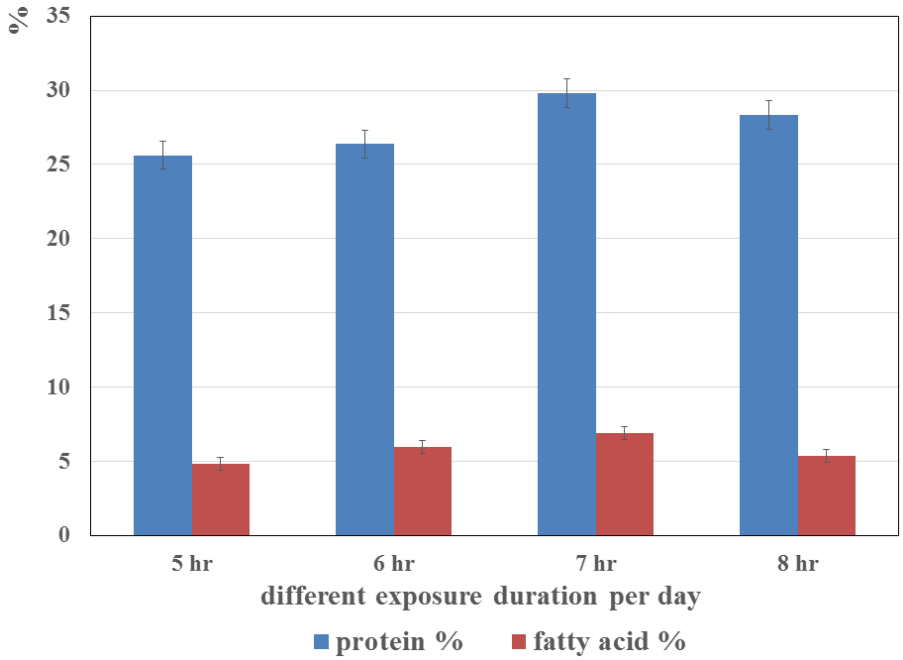

Figure 4.4 Protein percentage and fatty acid percentage in microalgae under different exposure duration, when exposure duration equals to 5, 6, 7, and 8 hrs.

Table 4.3 Fatty acid composition (%TFA, total fatty acid) of *S. dimorphus* under different exposure duration.

Fatty Acid	*Unit*	*5 hours/day*	*6hours/day*	*7hours/day*	*8hours/day*
C15:0	%TFA	1.1	0.7	0.9	0.7
C16:0	%TFA	13.5	15.8	17.9	15.7
C18:1cis	%TFA	12.3	14.8	13.5	17.0
C18:1trans	%TFA	1.6	1.3	8.3	1.3
C18:2	%TFA	11.7	11.9	12.0	10.1
C20:0	%TFA	0.3	0.7	0.1	0.2
C18:3	%TFA	20.7	21.8	22.0	18.2

4.2.1.2 Elemantal Analysis

Figure 4.5 and Figure 4.6 are the test results from elemental analyses. N% in Figure 4.5 will have the same trend of protein percentage depicted in Figure 4.4, since the nitrogen content multiplied by 6.25 is the way to calculate protein content. In this study, N% test and protein content test are done in different departments using the same sample; since they have same trend, the result is acceptable. N% under 7 hour/day exposure duration is slightly higher

than other exposure durations, as was the result for protein percentage. Hydrogen can exist in carbonhydrate, fatty acid and protein. H% indicates the efficiency of photosynthesis. In Figure 4.5, the H% does not show a significant difference among different exposure durations.

Sulfur is a component of protein such as Methionine and Met+cys and also can be a component of amino acids such as Methionine, Cysteine, Taurine and Lanthionine. The amino acids content test was not performed in this study. S% under different exposure shows no significant under different exposure duraions; it also varies. The C% is most like H%, since it can exist in carbonhydrate, fatty acid, and protein, most of the products of photosynthsis. So it generally indicates the efficiency of photosynthesis. C% under 7 hours/day shows a significant difference over other duration conditions, which indicates the biomass under this condition is higher than others, and the microalgae process photosynthesis occurs very well when the light supply is 7 hours daily.

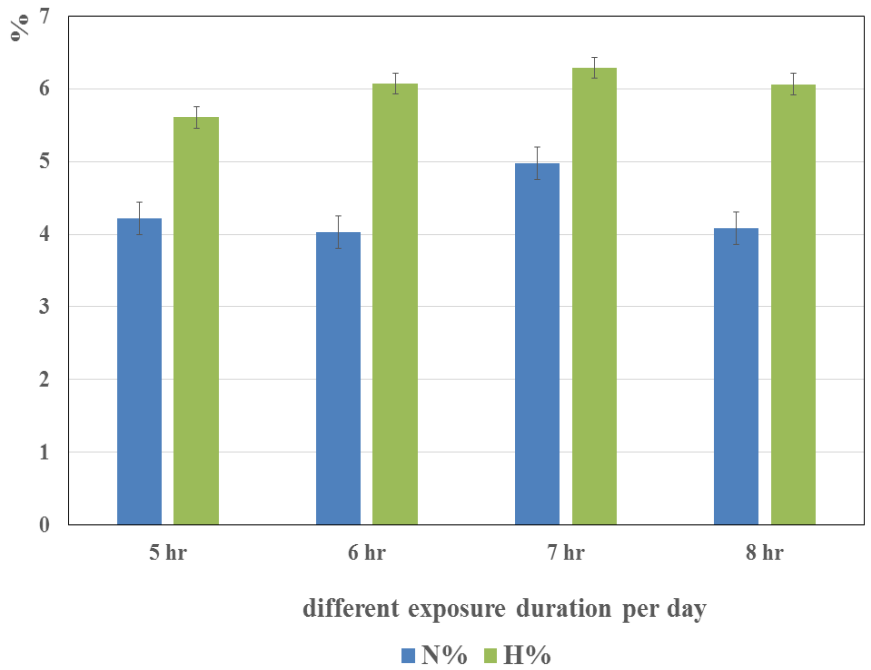

Figure 4.5. N% and H% in microalgae under different exposure duration, when exposure duration equals to 5, 6, 7, and 8 hrs.

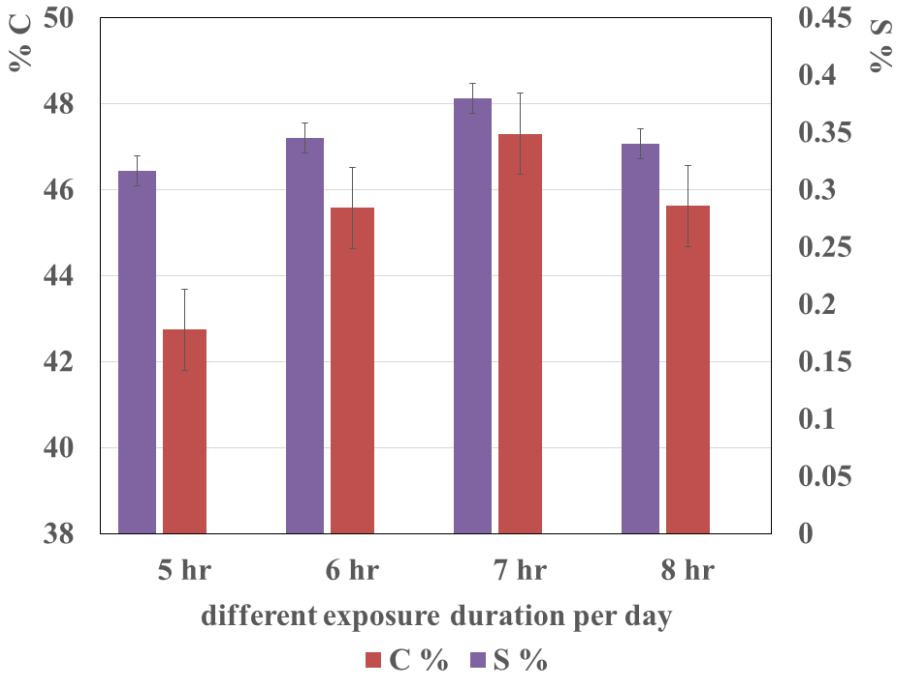

Figure 4.6. C% and S% in microalgae under different exposure duration, when exposure duration equals to 5, 6, 7, and 8 hrs.

Based on observations of the growth rate of microalgae under different light daily exposure durations, 8 hours/day, and 7 hours/day, 6 hours/day and 5 hours/day, the *S.dimorphus* grows well when daily light exposre duration is 7 hours. The cell density and cell productivity are obviously high, while protein content and fatty acid content are similar to other conditions. Thus, for the second state of this research, 7 hours/day will be used as the fixed exposure duration, to discover the optimal exposure frequency.

4.3 Cell Growth under Different Light Frequency

Changing pumping rate also changes the light exposure frequency for microalgae. The research tested exposure frequencies start around 70 cycles/hour, and 53 cycles/hour, 105 cycles/hour and 88 cycles were chosen as comparisons. Cell density and cell productivity

analyses under different light exposure frequencies are shown in Figure 4.7. As with testing duration, the dilution rate (0.1 day^{-1}) is used constantly in this whole research, so the areas of cell density and cell productivity are same even though the axis is different. When frequency is 53 cycles/hour, because of light limitation, the dark reaction of photosynthesis will be affected. When frequency reaches 70 cycles/hour, light supply is suitable for microalgae, and the cell density can be as high as 3.2 g/L and cell productivity can be 0.32 $g/L*day^{-1}$. After continued increases of the light frequency, the cell density and cell productivity drop because of photoinhibition (see Chapter II).

Cell density can be determined by counting microalgae cell number. Figure 4.8 shows the cell number counting results when the *S. dimorphus* reaches steady state under different exposure durations. The test is performed with a dry sample diluted into distilled water, and counting includes both live cells and dead cells. When the light frequency changes from 53 cycles/hour to 70 cycles/hour, cell density goes from 1.72×10^8 cells /mL to 1.91×10^8 cells/mL, and when frequency increases to 88 cycles/hour, the cell density drops to 1.75×10^8 cells/mL; when frequency is raised to 105 cycles/hour, the cell density goes lower, to 1.71×10^8. This is the same result trend shown in Figure 4.7. Thus, the limitation of light source will decrease the cell density of microalgae, while excessive light will cause photoinhibition and reduce biomass.

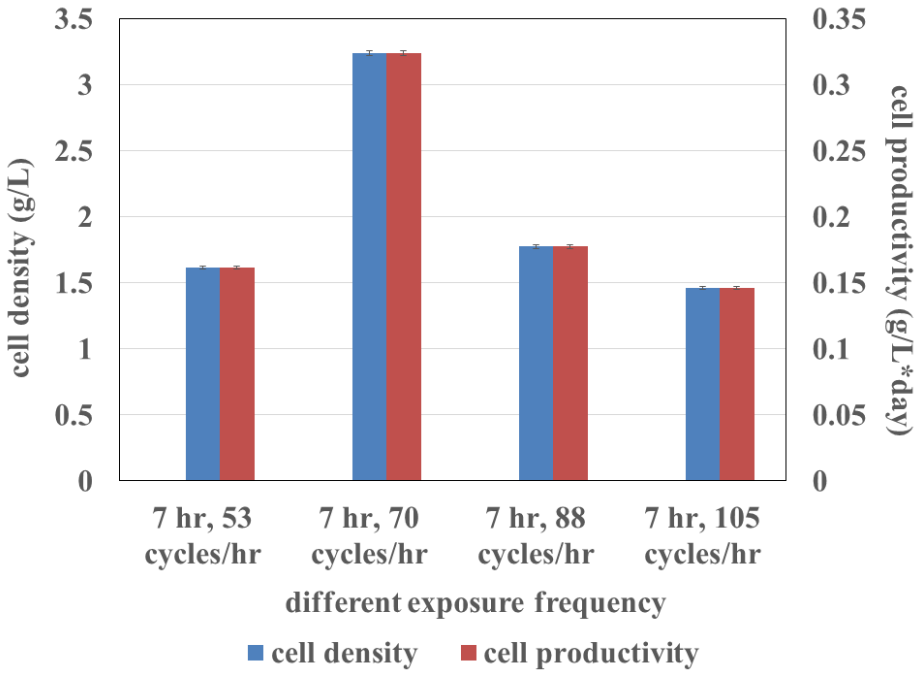

Figure 4.7. Cell density and cell productivity of microalgae under different exposure frequency, when exposure duration is 7 hrs/day.

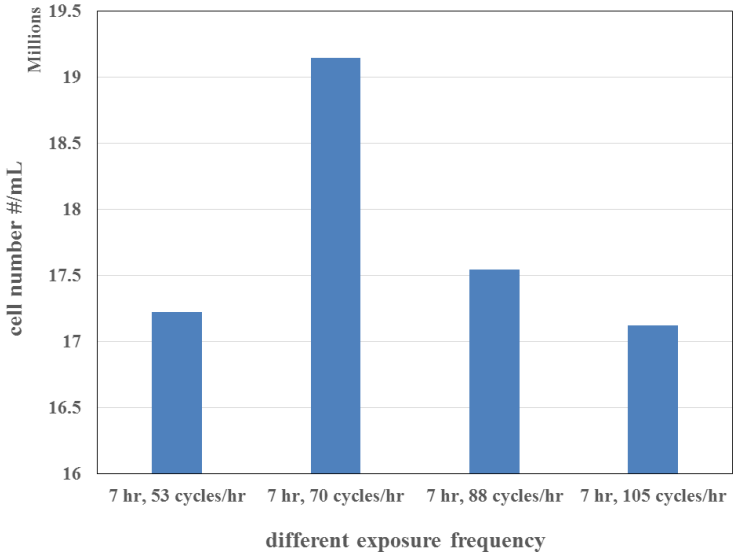

Figure 4.8. Microalgea cell number counting under different exposure frequency, when exposure duration is 7 hrs/day.

4.3.1 Biomass Analsyis under Different Light Frequency

4.3.1.1 Protein and Fatty Acids Analysis

As with biomass analysis when testing light duration, protein and fatty acids are two important contents in algal biomass. More efficient photosynthesis will produce more protein and fatty acid. Figure 4.9 below shows the protein and fatty acid percentage of *S. dimorphus*. The protein percentages under different frequencies are in the rage of 25% - 30%, a similar result to duration biomass protein analysis. Comparing different light frequency conditions, even the trend changes are not significant. The protein percentage under 70 cycles per hours exposure frequency shows a slightly higher result than under other duration conditions.

However, the fatty acids percentage of *S.dimorphus* under different exposure durations still shows little difference; the percentages of fatty acids are relatively similar in the frequency tests, as well. The fatty acid composition of *S. dimorphus* under different exposure frequencies is presented in Table 4.4 below. The percentage of elaidic acid (C18:1 cis) under 70 cycles/hour light frequency is significantly higher than other duration condition, but the remaining percentages of each individual fatty acid (% TFA, total fatty acid) are relatively similar to other conditions. Thus, under 70 cycles/hour exposure frequency, microalgae will produce more elaidic acid (C18:1 cis) than under other conditions.

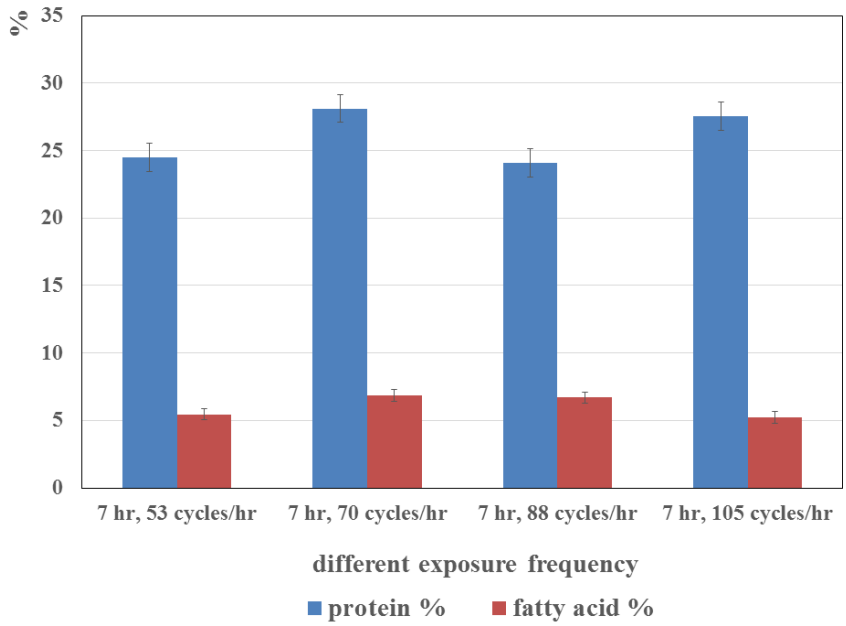

Figure 4.9. Protein percentage and fatty acid percantage of microalgae under different frequency, when exposure duration is 7 hrs/day.

Table 4.4 Fatty acid composition (%TFA, total fatty acid) of *S. dimorphus* at different exposure frequency, when exposure duration is 7 hours/day.

Fatty Acid	*Unit*	*7hr, 53cycles/hr*	*7hr, 70cycles/hr*	*7hr, 88cycles/hr*	*7hr, 105cycles/hr*
C15:0	%TFA	0.8	0.7	0.8	0.7
C16:0	%TFA	14.5	15.4	14.5	15.5
C18:1 cis	%TFA	14.5	17.8	14.5	13.6
C18:1 trans	%TFA	1.0	1.3	1.0	1.3
C18:2	%TFA	11.2	10.9	11.2	10.9
C20:0	%TFA	0.5	0.5	0.8	0.7
C18:3	%TFA	20.9	20.4	20.9	20.5

4.3.1.2 Element Content Analysis

Figure 4.10 and Figure 4.11 are the test results from elemental analysis. N% in Figure 4.10 will have the same trend of protein percentage shown in Figure 4.9, since the nitrogen content multiplied by 6.25 is the way to calculate protein content. N% and H% under different exposure light frequencies are relatively similar. Nitrogen and hydrogen molecules can exist in carbonhydrates, fatty acids, proteins and amino acids. Further, the hydrogen ion

will be produced in the first phase of photosynthesis. H % and N% can indicate the efficiency of photosynthesis.

As introduced in 4.2.1.2, sulfur exists in photosynthesis production, such as some kinds of protein and some amino acids. S% under 70 cycles/hour shows a slight difference compared to other exposure frequencies. When the frequency increases, the S% will decrease, but when the frequency is decreased, the S content is reduced, as well. The reason for this is photosynthesis efficiency. The C% under 70 cycles/hour as frequency shows a significant difference compared to other conditions, which means the biomass under this condition is high, and the microalgae process photosynthesis very well when light frequency is 70 cycles/hour.

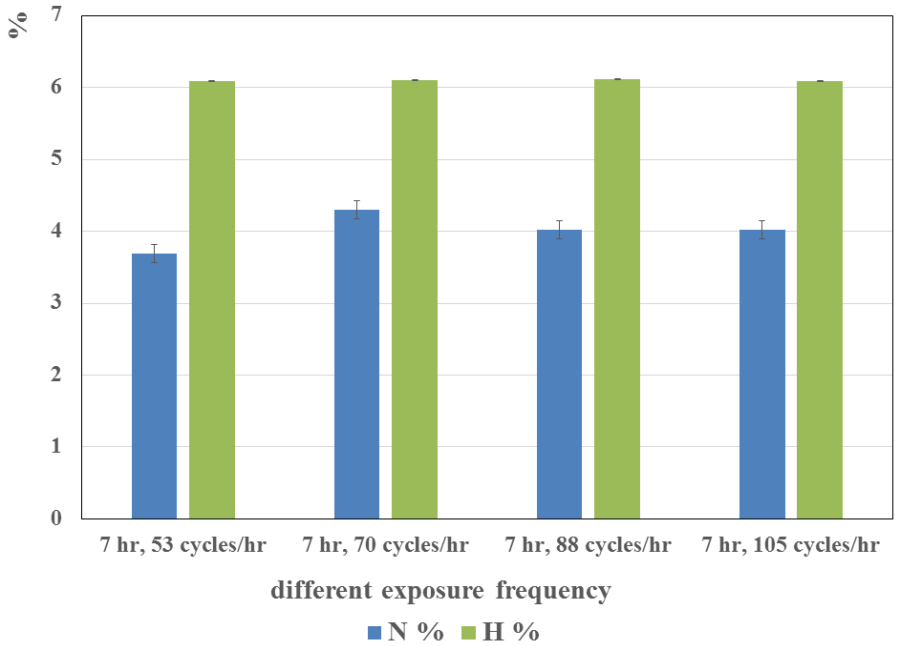

Figure 4.10. N % and H % of microalgae under different exposure frequency, when exposure duration is 7 hrs/day.

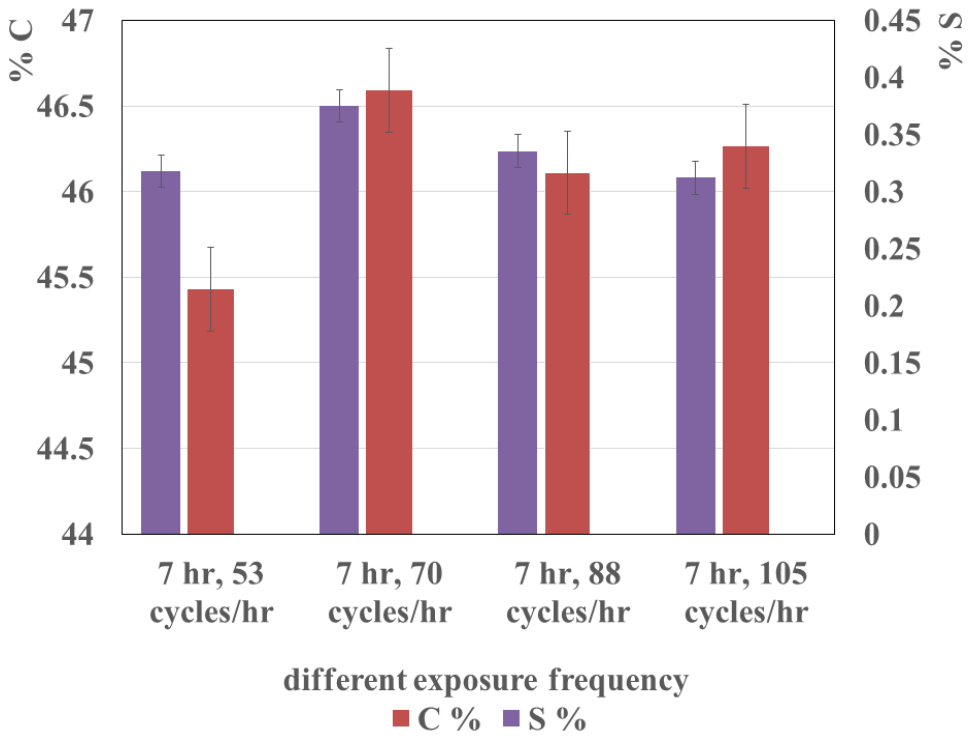

Figure 4.11. C% and S% of microalgae under different exposure frequency, when exposure duration is 7 hrs/day.

Chapter V

CONCLUSION

The fresh microalgae species *S. dimorphus* was used in this study to test optimal light exposure duration and exposure frequency in order to test the new two portion photobioreactor configuration. The statistical analysis indicates that light duration and light frequency will have a significant influence on growth rate of microalgae; mean values and standard error are from replicate samples.

Different daily exposure durations (8 hours, 7 hours, 6 hours, and 5 hours) and different exposure frequencies (53 cycles/hour, 70 cycles/hour, 88 cycles/hour, and 105 cycles/hour) were used during the test. The growth rates of micralgae under different light conditions were compared with cell density, cell productivity and cell number counting. As a result, when daily light duration is 7 hours and frequency is 70 cycles/hour, the growth rate is considerable for micalalge growth rate and protein content.

The productitvty of biomass was controlled by protein content, fatty acid content and elemental content analysis (C%, H%, N%, S%). The protein content results show when daily exposure duration is 7 hours and frequency is 70 cycles/hour, the protein percentage of biomass of microalgae is slightly higher than under other light conditions. It has potential ability to be animal feed since the considerable protein content. The fatty acid content analysis showed no significant difference between the different light exposure conditions. Even when comparing each fatty acid percentage of total fatty acid, some kinds of fatty acid under 7 hours daily duration or when frequency is 70 cycles/hour show significant differences compared to other light exposure conditions, but the rest of fatty acids are

somehow relatively the same or even lower. The result of element content analysis of C% shows a higher percentage when daily exposure duration is 7 hours and frequency is 70 cycles/hour. Nitrogen percentage has same result with protein content. C% and H% results showed no difference under different light exposure conditions. Also, the specific growth rate under this light exposure condition is 0.38 day -1 , while other research shows specific growth rate for the same microalgae species is 0.32 day-1 (Kang, 2012).

Overall, the concept of this two portion photobioreactor are apporaiate to produce microalgae biomass under certain light exposure durations and frequencies, It is a new approach to discover the light-dark cycle for microalgae growing. Protein content can be used in animal feed, while more research needed to be done to test the ability to produce biodiesel. What's more, to make sure this photobioreactor has a promising furture in real scale cultivation, more experiments are needed to be studied uncover the relationship between exposure duration and frequency.

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