CULTIVATION OF *ISOCHRYSIS MARITIMA* Billard and Parke FOR LIPID PRODUCTION USING AIRLIFT PHOTOBIOREACTOR

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By

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LIST OF ABBREVIATION

psi	: per square inch
kPa	: kilopascal
rpm	: rotation per minute
g	: gravity
v/v	: volume/volume
w/v	: weight/volume
vvm	: $volume_{air}/volume_{liquid}$/minute
HCl	: hydrochloric acid
$CHCl_3$	: chloroform
H_2SO_4	: sulphuric acid
MeOH	: methanol
(Na_2SO_4)	: disodium sulphate
FAME	: fatty acid methyl esther
PUFA	: polyunsaturated fatty acid
DHA	: docosahexanoic acid
EPA	: eicosahexanoic acid
ARA	: arachidonic acid
LA	: linoleic acid
ALA	: linolenic acid
NaCl	: sodium chloride

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LIST OF PUBLICATIONS AND SEMINARS

Seminars / Proceedings

i. Nik Farhana, N. N. M., Wan Maznah, W. O., Amirul, A. A., Yahya, A. R. M., (2010). The Effects of Heterotrophic Cultivation of *Isochrysis maritima* on Cell Biomass and Polyunsaturated Fatty Acid (PUFA) in Airlift Bioeactor. Proceeding in International Conference on Biotechnology and Food Engineering, WASET. Singapore.

ii. Nik Farhana, N. N. M., Wan Maznah, W. O., Amirul, A. A., Yahya, A. R. M., (2010). Heterotrophic Cultivation of *Pavlova* sp. in Airlift Bioreactor. Proceeding in the 7th IMT-GT Uninet & the 3rd Joint International PSU-UNS Conferences. Songkhla, Thailand.

iii. Nik Farhana, N. N. M., Wan Maznah, W. O., Amirul, A. A., Yahya, A. R. M., (2009). Unsaturated Fatty Acid Profiles of Marine Microalgae Isolated from Mukahead Coastal Water, Penang. Proceeding in the 7th Asia-Pacific Conference on Algal Biotechnology. New Delhi, India.

iv. Nik Farhana, N. N. M., Wan Maznah, W. O., Amirul, A. A., Yahya, A. R. M., (2009). Rapid *P*avlova Growth in Batch Heterotrophic Cultivation in an Airlift Bioreactor. Proceeding in the 7th Asia-Pacific Conference on Algal Biotechnology. New Delhi, India.

v. Nik Farhana, N. N. M., Wan Maznah, W. O., Amirul, A. A., Yahya, A. R. M., (2009). Preliminary Studies of Polyunsaturated Fatty Acids Detection in Microalgae. Proceeding in UNAIR – USM Second Collaborative Conference Life Sciences Synergy for Enhancement of Quality of Life. Surabaya, Indonesia.

PENGKULTURAN *ISOCHRYSIS MARITIMA* Billard dan Parke BAGI PENGHASILAN LIPID MENGGUNAKAN BEJANA ANGKUT UDARA BERCAHAYA

ABSTRAK

Isochrysis maritima telah dikulturkan di dalam bejana angkut udara bercahaya dengan kapasiti 4L dengan menyasarkan penghasilan biojisim, lipid dan asid lemak tidak tepu pelbagai jenis (PUFAs). Tiga jenis modus pengkulturan telah dijalankan bagi meningkatkan penghasilan biojisim dan lipid iaitu fotoautotrofik, heterotrofik dan fotoheterotrofik, melalui dua jenis pengubahsuaian pengkulturan iaitu pengkulturan kelompok berulang dan pengkulturan dua peringkat. Produktiviti lipid tertinggi telah dicapai melalui pengkulturan fotoheterotrofik iaitu1.39 g/L/hari, dengan 6.03 g/L/hari produktiviti biojisim. Pencapaian tersebut adalah 15-kali ganda lebih tinggi dari yang dicapai melalui pengkulturan fotoautotrofik (0.17 g/L/hari, 0.75 g/L/hari). Keputusan ini diperolehi melalui kombinasi pengkulturan strategi kelompok berulang dengan keamatan cahaya 156 μmol photon. Melalui kombinasi ini, hasil lipid yang dicatatkan adalah 3 kali ganda lebih tinggi ($Y_{p/fruc}$=0.18), berbanding pengkulturan strategi dua peringkat ($Y_{p/fruc}$ = 0.06). Peningkatan yang diperolehi ini telah menunjukkan keberkesanan bekalan cahaya dalam penghasilan lipid dalam kultur *I. maritima*. Kajian interaksi di antara metabolisma fotoautotrofik dan heterotrofik di dalam keadaan pengkulturan fotoheterotrofik pada keamatan cahaya yang rendah (45 μmol photon) telah menunjukkan kesan kehadiran fruktosa yang tidak ketara dalam kadar pertumbuhan fotoautotrofik. Walaubagaimanapun,

kadar pertumbuhan fotoautotrofik didapati sangat terencat dalam fotoheterotrofik pada keamatan cahaya tinggi (156 µmol photon). Oleh itu, pengkulturan fotoheterotrofik pada keamatan cahaya tinggi (156 µmol photon) telah dipilih sebagai pengkulturan terbaik untuk penghasilan lipid bagi *I. maritima*.

CULTIVATION OF *ISOCHRYSIS MARITIMA* Billard and Parke FOR LIPID PRODUCTION USING AIRLIFT PHOTOBIOREACTOR

ABSTRACT

Isochrysis maritima was cultivated in 4 L of airlift photobioreactor aiming for the production of high biomass, lipid and polyunsaturated fatty acid (PUFA)s production. Three types of cultivation modes have been applied to enhance the production of both biomass and lipid concentration which were photoautotrophic, heterotrophic and photoheterotrophic cultivation, employing two types of cultivation modification which were repeated-stage and two-stage cultivation strategy. The highest lipid productivity was achieved in photoheterotrophic cultivation condition which was 1.39 g/L/day with 6.03 g/L/day of biomass productivity. The achievement was approximately 15-fold higher than the concentration obtained from the photoautotrophic (0.17 g/L/day, 0.75 g/L/day). These outputs were accomplished with the combination of repeated-stage cultivation strategy at 156 µmol photon of light intensity. By using the combination, the lipid yield was found to be 3-fold higher ($Y_{p/fruc}$ = 0.18) than in the repeated-stage of heterotrophic cultivation condition ($Y_{p/fruc}$= 0.06). The improving results obtained have indicated the effectiveness of light supplementation in the lipid production of *Isochrysis maritima*. The interaction study between both heterotrophic and photoautotrophic metabolism in photoheterotrophic cultivation condition showed that at low light intensity (45 µmol photon), the presence of fructose or heterotrophic metabolism has no significant effect on photoautotrophic growth rate, but photoautotrophic growth was

highly inhibited in photoheterotrophic culture under high light intensity (156 μmol photon). Thus, repeated-batch cultivation strategy at high light intensity has been chosen as the best cultivation strategy in the enhancement of lipid production of *I. maritima*.

1.0 INTRODUCTION

Microalgae are photosynthetic organisms with simple requirements for growth like any other plants. Other than that, microalgae was also known can produce various valuable by-products in the form of proteins, pigments, biopolymers and carbohydrate such as docosahexanoic acid and carotenoid (Rawat *et al*., 2013). Presently, the main driving force for growing microalgae extensively is harvesting the lipid. Known for its potential as one of the promising feedstock for biodiesel production, microalgae has been massively cultivated with the aim for high lipid production. From the many sources of biodiesel, microalgal biodiesel seems promising in minimizing the production cost (Li *et al*., 2007).

Presently, various strategies have been suggested to enhance the production of both cell biomass and lipid. These include manipulation of the chemical parameters, physical parameters and cultivation modes (photoautotrophic, heterotrophic, mixotrophic and photoheterotrophic growth) (Chojnacka and Marquez-Rocha, 2004; Mata *et al*., 2010). Basically, microalgae were commonly grown in photoautotrophic condition, whereby both sunlight and carbon dioxide (CO_2) were employed as the main energy source of the cultivation. Although the lipid production was relatively high, this conventional cultivation method is frequently associated with a severe biomass production. Subsequently, the phenomenon contributes to a very low overall lipid productivity.

As often highlighted, lipid productivity takes into account both the lipid content within cells and the biomass produced by these cells and is therefore a reliable indicator of the potential lipid producer (Griffiths and Harrison, 2009; Brennan and Owende, 2010). Owing to the small sizes of microalgae coupled with

low biomass concentration, the harvesting process always posed a daunting task as this will also contribute to a higher downstream cost.

1.1 Motivation and scope of the project

Currently, numerous research efforts in microalgae and lipid production have been conducted in many parts of the world such as India, China and United States (Khan *et al*., 2009; Li *et al*., 2011; Menetrez, 2012). Very few, if any, have been conducted in Malaysia, focusing on high biomass and lipid production by using airlift photobioreactor. The inadequacy has open up an opportunity for us to investigate the potential of lipid production from locally isolated microalgae.

The present study focused on the cultivation and production of lipid by *Isochrysis maritima*, isolated from Penang Island coastal water of Malaysia. This genus was known as one of the high-lipid content microalgae. However, very limited research effort has been done using this strain. The major part of this thesis describes the production of *Isochrysis maritima* and its lipid using several cultivation conditions. The study was initiated with microalgal strain screening, involving seven species of locally-isolated microalgae from Penang island coastal water. A 4-liter airlift photobioreactor was employed to cultivate the microalgae, providing ample culture samples for lipid extraction purposes.

It is highly desirable to extensively explore and discover the potential of microalgae in biodiesel production by maximizing the yield and productivity of microalgal lipid. This study also sought out the metabolic interaction in photoheterotrophic cultivation condition as it has been reported to maneuver the production of the microalgae and its metabolites under this cultivation condition.

1.2 Problem statement

1. The production of lipid from microalgae is often associated with severely low lipid productivity due to low cell biomass or lipid production. Subsequently, the problem led to higher unit cost of downstream processes, which prohibit its commercialization.

1.3 Objectives of the study

1. To investigate the effect of medium modification on cell growth kinetics of *I. maritima*.
2. To determine the best cultivation strategy for the combination of cell growth, lipid and polyunsaturated fatty acids (PUFA) production.
3. To determine the effect of different light intensity and aeration towards the optimum lipid yield and productivity.

2.0 Literature Review

2.1 Algae

Algae are simple organisms that belong to a large, diverse group of organisms. They are defined as thallophytes, also known as primitive plants which lack true roots, stems, leaves, embryo and possess chlorophyll-a as the primary pigment (Brennan and Owende, 2010; Singh and Gu, 2010), which differentiates them from higher plants. According to Barsanti *et al*., (2008), the term „algae" refers to macroalgae (multicellular) and microalgae (unicellular), with the size ranging from 0.2-2.0 μm (diameter) for picoplankton to 60 m for giant seaweed (fronds length). Macroalgae are mainly cultivated along the shorelines and have long been used for production of phycocolloids like agar–agar, alginates or carrageenan (Pulz and Gross, 2004). However, microalgae are cultivated in closed culture systems known as photobioreactor and are extensively investigated for their byproducts (lipid, fatty acid and pigments).

Algae are mainly aquatic and are found almost everywhere from freshwater springs to marine habitat, with tolerance to a broad range of light intensity, pH, temperature, oxygen (O_2) and carbon dioxide (CO_2). They can be either benthic algae; attached to stone, mud or other plants, or planktonic algae that are suspended throughout the lighted regions in the water, which is the case of most of unicellular species (Barsanti, 2008). Most of the algae are solitary cells, some having the ability to move using flagella and reproducing vegetatively (cell division or cell fragmentation), asexually (production of spores), or sexually (plasmogamy, karyogamy or meiosis).

Algae generate an enormous fraction of the oxygen present in the atmosphere and a large quantity of organic carbon in the form of coal and petroleum. Owing to

their diversity, algae are divided into a few classes. The systematic classification of algae is primarily based on their pigment components (Harwood and Guschina, 2009). Prokaryotic members of this collection are grouped into two divisions, while eukaryotic members are grouped into nine divisions (Table 2.1).

Table 2.1: Classification of different algae groups (Andersen, 2004; Barsanti *et al*., 2008).

Kingdom	**Division**	**Class**
Prokaryote	Cyanophyte	Cyanophyceae
	Prochlorophyte	Prochlorophyceae
	Glaucophyte	Glaucophyceae
	Rhodophyte	Bangiophyceae
		Florideophyceae
	Heterokontophyte	Chrysophyceae
		Xanthophyceae
		Eustigmatophyceae
		Bacillarophyceae
		Raphidophyceae
		Dictyochophyceae
		Phaeophyceae
	Haptophyte	Pavlovophyceae
		Prymnesiophyceae
Eukaryote	Cryptophyte	Cryptophyceae
	Dinophyte	Dinophyceae
	Euglenophyte	Euglenophyceae
	Chlorarachniophyte	Chlorarachniophyceae
	Chlorophyte	Prasinophyceae
		Chlorophyceae
		Ulvophyceae
		Cladophorophyceae
		Bryopsidophyceae
		Zygnematophyceae
		Trentepohliophyceae
		Klebsormidiophyceae
		Charophyceae
		Dasycladophyceae

2.1.1 Microalgae

The majority of the algal organisms are dominated by microalgae which are composed of more than several thousands of unexplored species (Natrah *et al*., 2007). Microalgae are small (1-10 μm) and non-cohesive organism. They are mainly eukaryotic though prokaryotic cyanobacteria are frequently included into the microalgae groups due to the similarities between each other (Mutanda *et al*., 2011). Phycologists have categorized microalgae in a variety of classes, distinguished by their pigmentation, life cycle and basic cellular structure. It was suggested that the *Bacillariophyceae* (diatoms), *Chlorophyceae* (green microalgae) and *Chrysophyceae* (golden microalgae) represent the main important group amongst the microalgae classes due to their species abundance (Demirbas, 2010).

In recent decades, the biotechnology of microalgae has gained a lot of attention. Applications range from simple biomass production for food and feed to valuable products for ecological applications. The contributions of microalgae in aquaculture and health industries are widely known (Table 2.2). For these applications, the market is still developing and is expected to enhance by the employment of new biotechnological approach such as genetic modification of the microalgae.

To date, their applications have been extended into new areas, as an alternative to overcome the global shortages of fossil fuel. The current research has been focused on this topic and new species were explored substantially, with *Chlorella* and *Chlamydomonas* being the main workhorses (Figure 2.1).

Table 2.2: Microalgae with valuable products (Pulz, 2004; Mutanda *et al*., 2011; Larkum *et al*., 2012).

Microalgae	**Products**
Arthrospira platensis	Nutritional supplements
Crythecodinium sp.	Nutritional supplements, aquaculture feedstock, infants formula
Dunaliella salina	β-carotene
Haematococcus pluvialis	Carotenoids, astaxanthin
Isochrysis galbana	Aquaculture feedstock
Nannochloropsis sp.	PUFA
Pavlova lutheri	Nutritional supplements, aquaculture feedstock
Phaedactylum tricornutum	Nutritional supplements, aquaculture feedstock
Spirulina platensis	Phycocyanin, nutritional supplements, infants formula

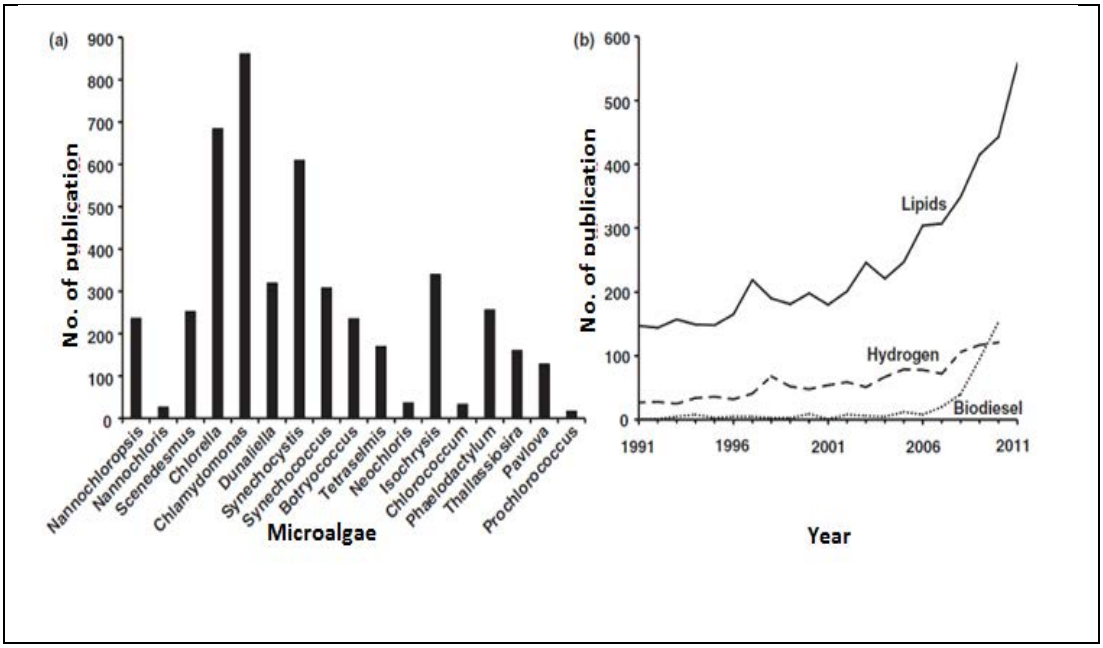

Figure 2.1: Statistics on microalgae-biofuels publications since 1991 (Larkum *et al*., 2012).

a) Publication of microalgal-biofuel based on strain;

b) Publication number by year for microalgae-biofuels.

2.1.2 *Isochrysis maritima*

Isochrysis is categorized as one of the haptophytes. *Isochrysis maritime* is rarely reported compared to the other phyla members such as *Isochrysis galbana* and *Pavlova* sp. (Andersen, 2004). Haptophytes are divided into two classes; Pavlovophyceae and Prymnesiophyceae, and this strain was classified under Prymnesiophyceae. As reported by Guiry and Guiry (2012), there are three species that have been taxonomically classified as *Isochrysis* which are *Isochrysis galbana*, *Isochrysis littoralis* and *Isochrysis maritima*. Due to their richness in carotenoid, the strain appears golden-brown in colour.

According to Billard and Gayral (1972), the young cells of the strain are usually non-motile and have a hemispherical (3 x 6μm) shape forming a cubic mass. As the cells approach maturity, they appear spherical (6 x 7μm) and encircled by concentric pectic envelopes. The strain reproduces asexually by division of the young non-motile cells and by production of elongated microtubules, with two homodynamic flagella. The mode of movement of this strain is highly specific. They move in a backward direction with rotation of the body around the long axis. Plate 2.1 showed the images of *I. maritima* used in the present study.

Owing to their richness in PUFA, *Isochrysis* sp. are becoming an important strain used in the aquaculture feedstocks (Table 2.2). The strain has been used as one of the main ingredients in an infant formula and health supplement in the form of biscuits enriched with EPA from the strain (Gouveia *et al*., 2008).

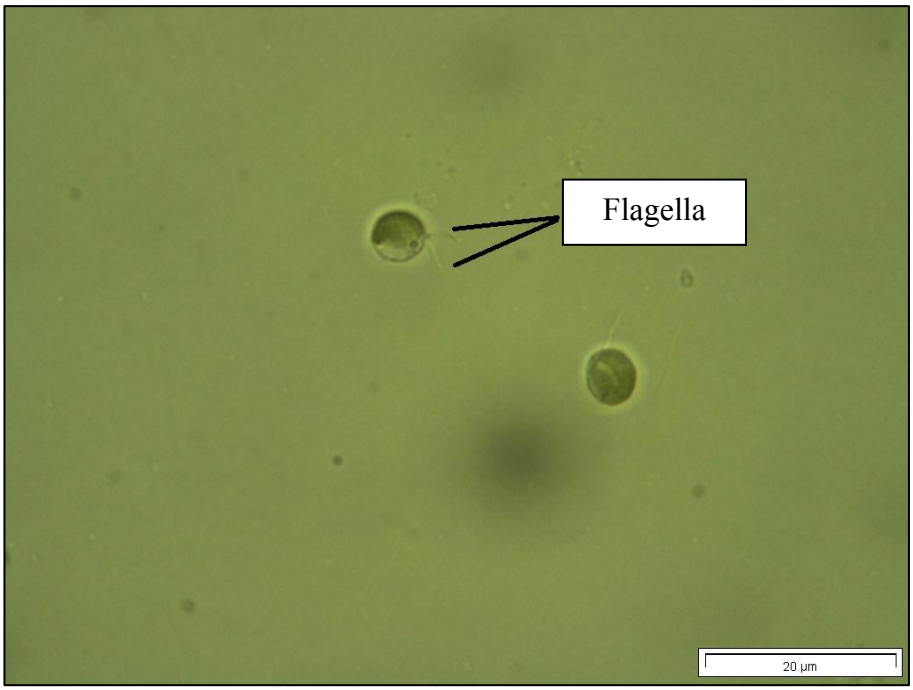

Plate 2.1: *Isochrysis maritima* using light microscope under 100x magnifications.

2.2 Biochemical composition of microalgae

Microalgae contain lipids and fatty acids as membrane components, storage products, metabolites and sources of energy. In living organisms, lipids serve as storage material in the cell, structural components of cellular membranes and participate in signaling pathways (Fahy *et al*., 2011). On the other hand, fatty acids are the building blocks of various types of lipid.

2.2.1 Lipids

Lipids are a diverse and ubiquitous group of compounds which serves several key biological functions in organisms. The term „Lipid" is often loosely used to describe any group of compounds that are insoluble in water but soluble in nonpolar solvents such as benzene, ether and chloroform (Bailey and Ollis, 1986).

According to Christie (2011) and Fahy *et al*., (2011), lipids are classified into simple and complex groups. Simple lipids are those yielding, at most, two types of primary product per mole upon hydrolysis, mainly consist of fatty acid, glycerol (triacylglycerol; TAG, diacylglycerol;DAG), sterols and wax ester. As depicted in Figure 2.2, triacylglycerol is a glycerol esterified with three fatty acids, and in the presence of alcohol, TAG reacts to form biodiesel with glycerol as a by-product (Suali and Sarbatly, 2012). Hence, TAG is the main material in the production of biodiesel (Huang *et al*., 2010). On the other hand, complex lipids are those yielding three or more hydrolysis products per mole, constituted of phospholipids and glycolipids.

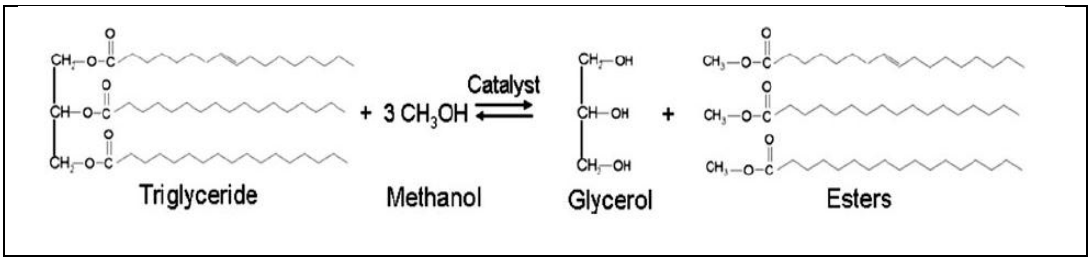

Figure 2.2: Transesterification reaction of TAG extracted from microalgal oils for fatty acid methyl ester (biodiesel) production (Gong and Jiang, 2011).

Microalgae are recognized as one of the good lipid producer among other microorganisms. Owing to their vast biodiversity, the lipid content and productivity of the microalgal differ tremendously. The values of lipid content reported ranges from of the low 10 to 30 %up to 80% of the dry cell weight (Larkum *et al*., 2012). Table 2.3 shows lipid content and lipid productivity of various species of microalgae.

The accumulation of lipid in microalgae cells depends on various factors. These include growth temperature, pH, nutrients, growth condition (photoautotrophic, heterotrophic, photoheterotrophic or mixotrophic), harvesting time and the strain (Perez-Garcia *et al*., 2011). According to Hu *et al*., (2008), the synthesis and accumulation of large amounts of TAG occur in the cell when the microalgae are placed under stress conditions imposed by chemical or physical environmental stress, either acting individually or in combination. These stress conditions can alter the normal metabolic pathways of cells, causing a metabolic preference from carbohydrate or protein to lipid synthesis. Amongst the stress employed, high light exposure and nutrient limitation are the most effective strategy in lipid accumulation (Packer *et al*., 2011; Go *et al*., 2012). These strategies are frequently applied in microalgae cultivation producing lipid and polyunsaturated fatty acids (PUFA).

Table 2.3: Lipid content and lipid productivity of various species of microalgae (Rodolfi *et al*., 2009; Mata *et al*., 2010).

Microalgae strains	Lipid content (% biomass)	Lipid productivity (mg/L/d)
Chaetoceros calcitrans CS 178	39.8	17.6
Chaetoceros muelleri F&M-M43	33.6	21.8
Chlorella sorokiniana IAM-212	19.3	44.7
Chlorella sp. F&M-M48	18.7	42.1
Chlorella vulgaris CCAP 211/11b	19.2	32.6
Chlorella vulgaris F&M-M49	18.4	36.9
Chlorococcum sp. UMACC 112	19.3	53.7
Ellipsoidion sp. F&M-M31	27.4	47.3
Isochrysis sp. (T-ISO) CS 177	22.4	37.7
Isochrysis sp. F&M-M37	27.4	37.8
Monodus subterraneus UTEX 151	16.1	30.4
Nannochloropsis sp. CS 246	29.2	49.7
Nannochloropsis sp. F&M-M24	30.9	54.8
Nannochloropsis sp. F&M-M26	29.6	61
Nannochloropsis sp. F&M-M27	24.4	48.2
Nannochloropsis sp. F&M-M28	35.7	60.9
Nannochloropsis sp. F&M-M29	21.6	37.6
Neochloris oleobundans	29-65	90-134
Pavlova lutheri CS 182	35.5	50.2
Pavlova salina CS 49	30.9	49.4
Phaeodactylum tricornutum F&M-M 40	18.7	44.8
Porphyridium cruentum	9.5	34.8
Scenedemus F&M-M19	19.6	40.8
Scenedemus quadricauda	18.4	35.1
Scenedemus sp. DM	21.1	53.9
Skeletonoma costatum CS 181	21	17.4
Skeletonoma sp. CS 252	31.8	27.3
Tetraselmis suecica F&M-M33	8.5	27
Tetraselmis suecica F&M-M35	12.9	36.4
Tetraselmis sp. F&M-M34	14.7	43.4
Thalassioria pseudonana CS 173	20.6	17.4

2.2.2 Fatty acid

Fatty acids are classified based on the number of carbon chain length, namely medium-chain length (MC; C10–C14), long-chain length (LC; C16–C18) and very long-chain length (VLC; ≥ C20). They can be saturated or unsaturated with varying number and position of the double bonds on the carbon chain backbone (Hu *et al*., 2008). Saturated fatty acids (SFA) contain no double bonds and mainly predominate in a form of palmitic acid (C16:0) and myristic acid (C14:0). In constrast, monounsaturated fatty acids (MUFA) have one double bond, primarily present as palmitoleic acid (C16:1) and oleic acid (C18:1). According to Basova (2005), SFA and MUFA make up of approximately 2.1-58.9% of the total fatty acid presence in microalgae, depending on the genetic inheritance of the microalgae species (Ratledge, 2004).

Polyunsaturated fatty acids (PUFA)s contain two or more double bonds in their carbon backbone. VLC-PUFAs, namely, docosahexanoic acid (DHA; C22:6n3), eicosapentaenoic acid (EPA; C20:5n4), arachidonic acid (AA; C20:4n6), linolenic acid (ALA; C18:3n6) and linoleic acid (LA; C18:2n6) are known to have a wide range of pharmaceutical applications in human and marine animals. They have been widely used in prevention and treatment of various diseases such as heart and inflammatory diseases in humans and also as the health supplement for physiological development of marine animals.

Initially, PUFAs are widely obtained from fish oil. However, in recent years, problem arise with its unpleasant odor, high purification cost and poor oxidative stability, accompanied by inadequate fish supplies making it less favorable (Li *et al*., 2005). Microalgae have been known as a good PUFAs producer. In comparison to

fish, microalgae can directly produce fatty acid, making the process simple and economical (Harun *et al*., 2010).

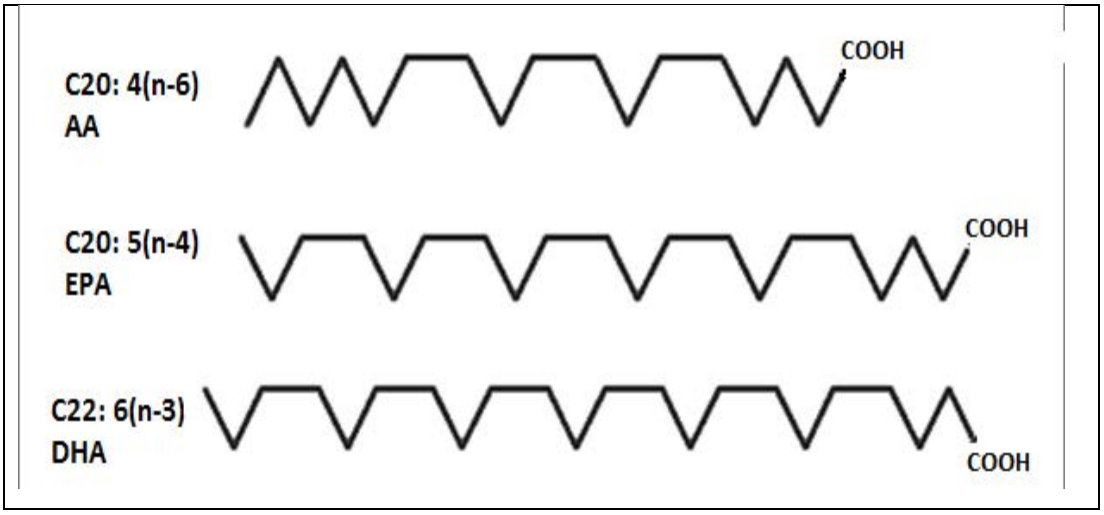

Figure 2.3: PUFAs of high pharmaceutical and nutritional value (Pulz and Gross, 2004).
AA; arachidonic acid; EPA; eicosapentaenoic acid; DHA; docosahexanoic acid.

2.2.3 General biosynthesis of lipid and fatty acid

The biosynthesis of lipid in microalgae was first described using *Chlamydomonas reinnhardtii.* Among the microalgal strain, *C. reinhardtii* has been a model organism for many fundamental biological processes and metabolisms study (Li-Beisson, 2012). It is generally believed that the basic pathways of lipid and fatty acid in microalgae are similar with higher plants. However, it has been poorly studied compared to higher plants. Figure 2.4(a) shows a modified illustration on basic overview of fatty acid and lipid biosynthesis pathway in microalgae.

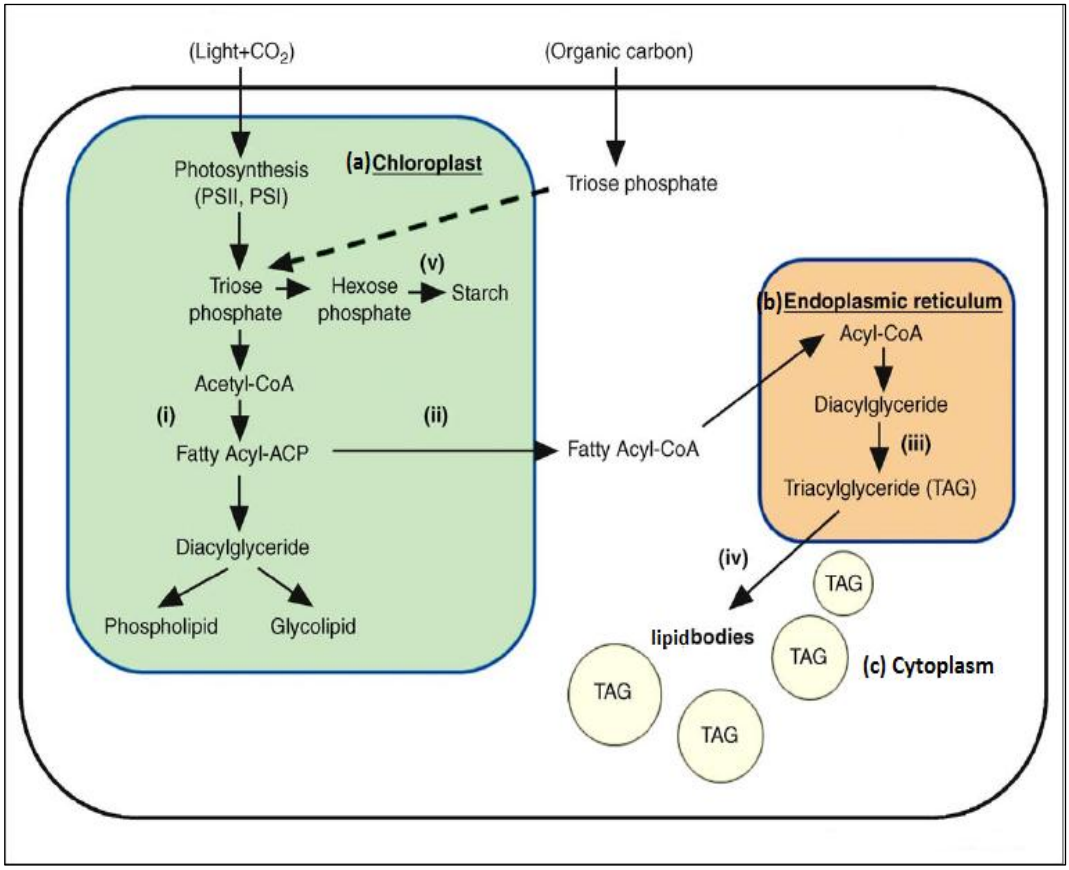

Figure 2.4(a): Illustration on basic overview of pathway in fatty acid and lipid biosynthesis (Scott *et al*., 2010).

(i): acetyl-CoA carboxylase; (ii): fatty acid thioesterases; (iii): TAG biosynthesis enzyme, including acyl-CoA:diacylglycerol acyltransferase (DGAT);

(iv): lipid body formation; (v): ADP-glucose pyrophosphorylase and starch synthesis

The synthesis routes of lipid in microalgae may consist of the following three steps; (i) the biosynthesis of fatty acid; (ii) the biosynthesis of TAG; and (iii) the discharge of TAG in a form of lipid bodies. In microalgae, the complete pathway from carbon fixation to lipid formation occurs in the single cell, whereas the synthesis and accumulation of lipid in higher plant only occur in specific tissues or organs.

i) Biosynthesis of fatty acid

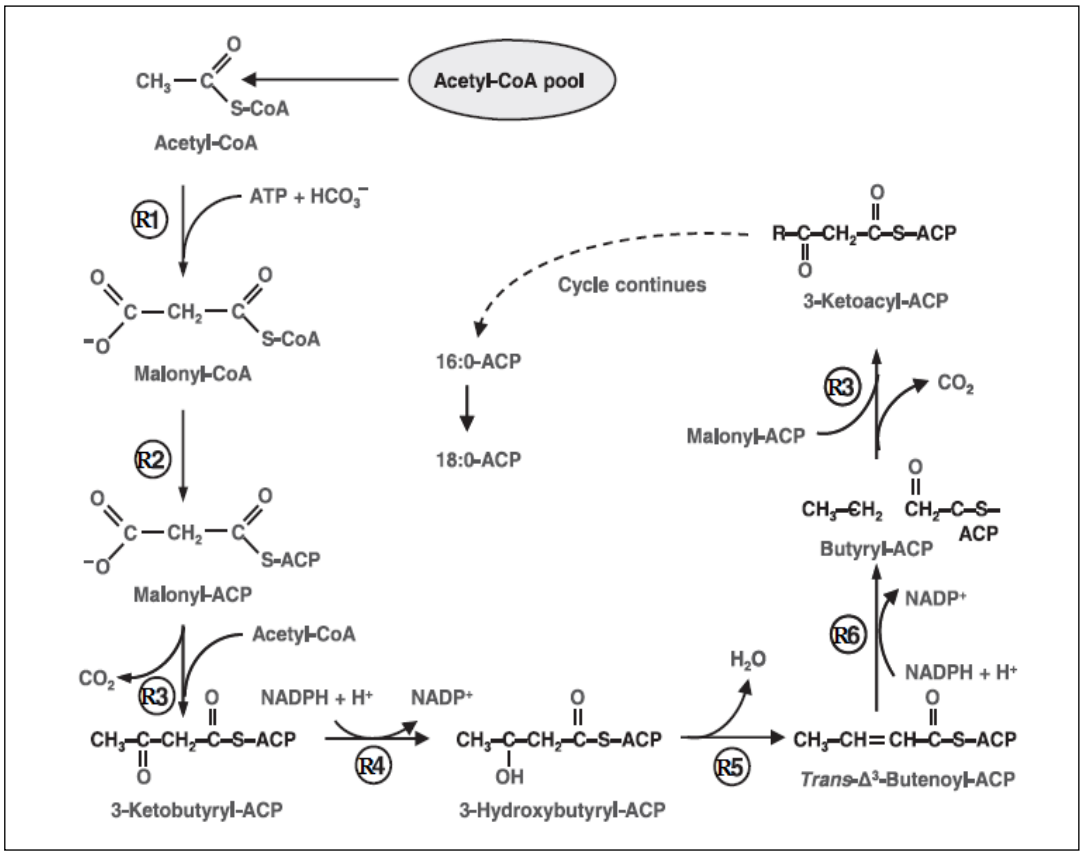

Figure 2.4(b): Fatty acid *de novo* synthesis pathway in microalgae (Hu *et al*., 2008)

R1: acetyl CoA carboxylase; R2: malonyl CoA:ACP transferase; R3: 3-ketoacyl synthase;
R4: 3-ketoacyl-ACP reductase; R5: 3-hydroxylacyl ACP dehydrase; R6: enoyl ACP reductase

The metabolic pathway of fatty acid synthesis (shown in Figure 2.4b) is also known as *de novo* synthesis and occurs primarily in chloroplast. In fatty acid synthesis, acetyl CoA carboxylase (ACCase) serves as the main regulatory enzyme that controls the rate of fatty acid synthesis (Livne and Sukenik, 1990). The ACCase is generated by the conversion of glycosis-derived pyruvate and acts as the substrate for acetyl CoA. It catalyses the first reaction in the pathway by catalyzing the conversion of acetyl CoA to malonyl CoA. The transformation of malonyl CoA to malonyl ACP in the second reaction is considered as one of the important reactions in the pathway. The malonyl ACP produced serves as the central carbon donor for

the subsequent fatty acid elongation process. The elongation process of fatty acid starts with a series of condensation process (R3), accompanied by three additional process; reduction (R4 and R6) and dehydration (R5). At the end of these four reactions, saturated C16-ACP or C18-ACP fatty acid or both are produced.

The biosynthesis of VLC-PUFAs in microalgae utilizes iterative desaturation and elongation process. Figure 2.4(c) shows the elongation and desaturation process of fatty acid to form VLC-PUFAs.

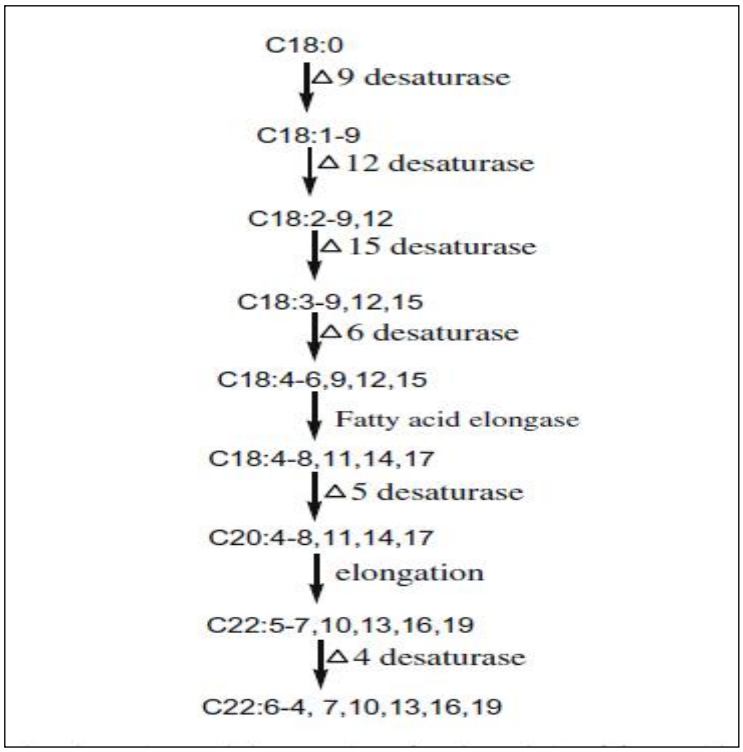

Figure 2.4(c): The elongation and desaturation of carbon chain of fatty acids in microalgae (Huang *et al*., 2010).

The desaturation process of carbon chain of fatty acid at C18 starts by the introduction of cis-double bond at the specific position in the fatty acid chain (Khozin-Goldberg *et al*., 2011). Further elongation process takes place to produce a VLC fatty acid (≥ 20C) (Huang *et al*., 2010). The elongation process continues until the acyl group is removed from the ACP which indicates the termination of the

subsequent elongation process (Ohlrogge and Browse, 1995). The synthesized fatty acids are transferred from the chloroplast to the endoplasmic reticulum (ER) for the synthesis of membrane complex lipid and cytosolic simple lipid (mainly TAG). The simplified schematic diagram of TAG biosynthesis pathway is shown in Figure 2.6(d).

ii) Biosynthesis of lipid (TAG)

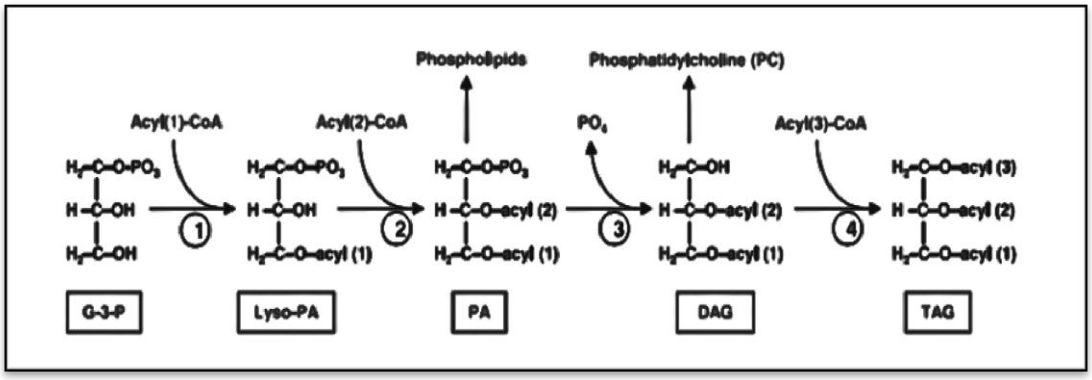

Figure 2.4(d): Simplified schematic diagram of triacylglycerol (TAG) biosynthesis pathway in microalgae (Hu *et al*., 2008).

G-3-P: glycerol-3-phosphate; Lyso-PA: lysophosphatidic acid; PA: phosphatidic acid; DAG: diglyceride; TAG: Triacylglycerol

Generally, G-3-P and acetyl CoA are two major primers in the biosynthesis of TAG. The synthesized fatty acid (fatty acyl CoA) reacts with one hydroxyl group of G-3-P, resulting in the formation of lyso-PA which later combines with another acetyl CoA to form PA. These two reactions are catalyzed by glycerol phosphate acyl-transferase. This is followed by PA being hydrolyzed by phosphatidate phosphatase to form DAG. Subsequently, DAG combined with the third acetyl-coA

to complete the biosynthesis of TAG, catalyzed by glyceryl diester transacylase (Hu *et al*., 2008; Huang *et al*., 2010). TAGs are release to the cytoplasm in a form of lipid bodies.

2.3 Microalgae cultivation modes

The growth characteristic and biochemical composition of microalgae is generally dependent on cultivation modes. There are four major types of cultivation modes: photoautotrophic, heterotrophic, photoheterotrophic and mixotrophic (Chen *et al*., 2011*)*. The biomass and lipid productivities of different microalgae species under different cultivation modes are shown in Table 2.4.

Table 2.4: The biomass and lipid productivity of different microalgae species under different cultivation conditions from recent publications.

Cultivation condition	Algal strain	*P** biomass (g/L/day)	*P** Lipid (g/L/day)	References
Photoautotrophic	*Botryococcus braunii* UTEX57	0.03	5.5	Yoo *et al*., (2010)
	Chaetoceros calcitrans CS178	0.04	17.6	Rodolfi *et al*., (2009)
	Chlorella sorokiniana IAM-212	0.23	44.7	Rodolfi *et al*., (2009)
	Chlorella sp. F&M-M48	0.23	41.2	Rodolfi *et al*., (2009)
	Chlorella vulgaris#259	0.01	38.0	Liang *et al*., (2009)
	Chlorella vulgaris INETI 58	0.18	7.4	Gouveia & Oliveira (2009)
	Chlorella vulgaris F&M-M49	0.20	36.9	Rodolfi *et al*., (2009)
	Chlorella vulgaris	0.133	0.04	Heredia-Arroyo *et al*., (2011)
	Chlorococcum sp. UMACC	0.28	53.7	Rodolfi *et al*., (2009)
	Dunaliella tertiolecta IPIMAR	0.12	20.0	Gouveia & Oliveira (2009)
	Ellipsoidion sp. F&M-M31	0.17	47.3	Rodolfi *et al*., (2009)
	Isochrysis sp. (T-ISO) CS 177	0.17	37.7	Rodolfi *et al*., (2009)
	Isochrysis sp. F&M-M37	0.14	37.8	Rodolfi *et al*., (2009)
	Monodus subterraneus	0.19	30.4	Rodolfi *et al*., (2009)

	UTEX151			
	Nannochloropsis sp. CS246	0.17	49.7	Rodolfi *et al*., (2009)
	Nannochloropsis oculata	-	0.32	Su *et al*., (2011)
	Tetraselmis sp. F&M-M34	0.3	43.4	Rodolfi *et al*., (2009)
Heterotrophic	*Chlorella protothecoides*	4.0-4.4	1.88	Cheng *et al*., (2009)
	Chlorella vulgaris#259	0.15	35	Liang *et al*., (2009)
	Chlorella protothecoides	0.99	0.25	Heredia-Arroyo *et al*., (2010)
	Chlorella vulgaris	0.25	0.08	Heredia-Arroyo *et al*., (2011)
	Chlorella zofingiensis	-	0.31	Liu *et al*., (2011)
	Chlorella protothecoides	1.36	0.19	Heredia-Arroyo *et al*., (2010)
Mixotrophic	*Chlorella vulgaris* #259	0.25	54	Liang *et al*., (2009)
	Scendesmus obliquus	0.51	58.6	Mandal and Mallick (2009)
	Chlorella protothecoides	1.59	0.27	Heredia-Arroyo *et al*., (2010)
	Chlorella sorokiniana	0.11	0.69	Wan *et al*., (2011)
	CCTCCM209220			
	Chlorella vulgaris	1.62	0.25	Heredia-Arroyo *et al*., (2011)
	Dunaliella salina	0.04	0.16	Wan *et al*.,

	FACHB435			(2011)
	Nannochlropsis oculata CCMP525	0.05	0.19	Wan *et al*., (2011)
	Pseudochlorococcum sp.	0.76	0.29	Li *et al*., (2011)
	Chlorella sp.	0.45	0.11	Cheirsilp & Torpee (2012)
	Chlorella vulgaris	2.5	1.1	Mitra *et al*., (2012)
	Nannochloropsis sp.	0.59	0.15	Cheirsilp & Torpee (2012)
	C. vulgaris	0.25	0.054	Liang *et al*., (2009)
Photoheterotrophic	*C. minutissima* UTEX2341	1.78	0.29	Li *et al*., (2011)
	C. minutissima UTEX2341	0.05	0.6	Yang *et al*., (2011)
	C. vulgaris ESP-31	nd	0.115	Yeh & Chang, (2012)

*P** : productivity

2.3.1 Photoautotrophic

Photoautotrophic is the most employed cultivation modes in microalgae production. It is primarily employed to enhance light-regulated metabolites in microalgae. In photoautotrophic culture, the cells harvest light as an energy and utilize CO_2 as the main carbon source. Due to the elimination of organic carbon substrates, phototoautotrophic offers lower production cost than other cultivation condition. Moreover, the contamination problem is less severe, which makes it as the preferred condition for open pond cultivation condition (Chen *et al*., 2011).

However, photoautotrophic condition severely limits the cell biomass production due to cellular self-shading that limits light penetration at high cell densities towards the end of the cultivation period (Cheirsilp and Torpee, 2012). Consequently, the low cell biomass concentration obtained will raise the biomass harvesting cost, which is one of the main concerns in microalgae cultivation.

2.3.2 Heterotrophic

Some of the microalgae have the ability to grow heterotrophically, utilizing organic carbon as the sole energy and carbon source for growth (Mata et al., 2010). Due to the absence of light, heterotrophic is known to be a cost-effective and also an alternative method for a mass cultivation of microalgae. In comparison to photoautotrophic, heterotrophic takes advantage of fast growth, high production rate, and convenient harvesting process (Zheng *et al*., 2012).

Nevertheless, heterotrophic have several major limitations that should be taken into consideration. Table 2.5 shows several major limitation of heterotrophic cultivation condition.