PRODUCTION OF OMEGA-3 FATTY ACID FROM *NANNOCHLOROPSIS* SP. ON THE EFFECTS OF CARBON DIOXIDE AND LIGHT INTENSITY

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SUPERVISOR DECLARATION

I hereby declare that I have checked this thesis and in my opinion, this thesis is adequate in terms of scope and quality for the award of degree of Bachelor of Chemical Engineering (Biotechnology).

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I hereby declare that the work in this thesis entitle by **"Production of Omega-3 Fatty Acids From *Nannochloropsis* sp. on the Effect of Carbon Dioxide and Light Intensity"** is my own except for quotations and summaries which have been duly acknowledged. The thesis has not been accepted for any degree and is not currently submitted for award of other degree.

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ABSTRACT

Omega-3 polyunsaturated fatty acid (PUFA) plays a vital role in a number of human health aspects substantially for the regulation of biological function, prevention and treatment of human diseases. Subsequently, humans cannot synthesize the omega-3 PUFA, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), thus, intake comes predominantly from dietary. Therefore, the microalgae was discovered as an alternative source of fatty acids compared to fish oil since it offers better purification essential and has no risk of chemical contamination. Moreover, it also acts as a carbon dioxide fixer by photosynthesis process in reducing the atmospheric carbon dioxide. There are major factors give affect for microalgae growth which is carbon source, irradiance level, temperature, nutrient availability and salinity. This study is carried out to investigate carbon dioxide effect in the specific range of 2%, 5%, 10% and 15% at flow rate of 0.2 L/min. Meanwhile, the irradiance levels are examined by using 31 µmol photons m^{-2} s-^{1}, 82 µmol photons m^{-2} s^{-1} , 125 µmol photons m^{-2} s^{-1} and 156 µmol photons m^{-2} s-^{1} by applying one factor at time (OFAT) in maximizing the production of omega-3 fatty acids. The microalgae of *Nannochloropsis* sp. are cultured in f/2 medium with 300 ml working volume for 7 days of cultivation in an experimental system of photobioreactor. The omega-3 fatty acid compositions of *Nannochloropsis* sp. under different culture conditions are analyzed by lipid extraction and gas chromatography analysis in order to acquire the maximum amount of yield production. The results showed that the highest eicosapentaenoic acid productivity by *Nannochloropsis* sp. had been obtained at 2% of carbon dioxide aeration and 156 µmol photons m^{-2} s^{-1} which are 31.3121 mg/ml and 35.1339 mg/ml, respectively. The fatty acid accumulation of *Nannochloropsis* sp. could be prevailed to produce a high yield under their optimal conditions which are in maximal efficiency of carbon dioxide and high of light intensity.

ABSTRAK

Asid lemak omega-3 memainkan peranan penting dalam beberapa aspek kesihatan terutamanya untuk peraturan fungsi biologi, pencegahan dan rawatan penyakit. Selain itu, manusia tidak boleh mensintesiskan asid lemak omega-3 iaitu, asid eicosapentaenoik dan asid docosahexaenoik, oleh itu, pengambilan asid lemak omega-3 ini diperolehi daripada pemakanan. Disamping itu, mikroalga ditemui sebagai sumber alternatif asid lemak yang penting berbanding minyak ikan kerana ia mempunyai kandungan yang lebih asli dan tidak mempunyai risiko pencemaran kimia. Ia juga bertindak sebagai pengguna karbon dioksida yang berkesan melalui proses fotosintesis bagi mengurangkan kandungan karbon dioksida dalam atmosfera. Terdapat beberapa faktor utama yang memberikan kesan terhadap pertumbuhan mikroalga antaranya ialah sumber karbon, tahap kepekatan cahaya, suhu, nutrien dan kadar kepekatan garam. Kajian ini dijalankan untuk mengkaji kesan parameter karbon dioksida dalam julat peratusan 2%, 5%, 10% dan 15% pada kadar aliran 0.2 L/min. Sementara itu, kesan kepekatan cahaya diuji terdiri daripada 31 µmol photons $m^{-2}s^{-1}$, 82 µmol photons $m^{-2}s^{-1}$, 125 µmol photons $m^{-2}s^{-1}$ dan 156 µmol photons m^{-2} s^{-1} dengan mengaplikasikan satu faktor pada satu masa dalam memaksimumkan pengeluaran asid lemak omega-3. *Nannochloropsis* sp. diternak dalam medium f/2 sebanyak 300 ml selama 7 hari dalam sistem eksperimen photobioreaktor. Komposisi asid lemak omega-3 dalam *Nannochloropsis* sp. di bawah keadaan persekitaran yang berbeza dianalisis melalui kaedah pengekstrakan lipid serta analisis kromatografi gas untuk menentukan hasil pengeluaran yang maksimum. Keputusan menunjukkan bahawa produktiviti tertinggi kandungan asid lemak omega-3 *Nannochloropsis* sp. dihasilkan pada 2% daripada pengaliran karbon dioksida sebanyak 31.3121 mg/ml manakala, 35.1339 mg/ml dihasilkan bagi kesan pengcahayaan pada 156 µmol photons $m^{-2}s^{-1}$. Pengeluaran asid lemak omega-3 yang tinggi daripada *Nannochloropsis* sp. dapat dihasilkan di bawah keadaan optimum iaitu dalam kecekapan maksimum karbon dioksida dan keamatan cahaya yang tinggi.

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PUFAs	Polyunsaturated fatty acids
EPA	Eicosapentaenoic acid
DHA	Docosahexaenoic acid
ALA	α-linolenic acid
LA	Linoleic acid
AA	Arachidonic acid
ω	Omega
GRAS	Generally Regarded as Safe
PCBs	Polychlorinated biphenyls
CO_2	Carbon dioxide
GHGs	Green House Gases
sp.	Species
µmol photons m^{-2} s^{-1}	Micromol photons per square meter per second
v/v	Volume per volume
C	Carbon
H	Hydrogen
O	Oxygen
-COOH	Carboxyl group
MUFAs	Monounsaturated fatty acids
LPS	Lipopolysaccharide
PGs	Prostaglandins
LTs	Leukotrienes
NH^{4+}	Ammonium ion
NO_3	Nitrate ion
PO_4^{3-}	Phosphate ion
g	Gram
mg	Milligram
NaCl	Sodium chloride
KCl	Potassium chloride
tris-base	2-amino-2-hydroxymethyl-1,3-propanediol
$MgSO_4.7H_2O$	Magnesium sulfate heptahydrate
$CaCl_2.2H_2O$	Calcium Chloride Dihydrate
$NaHCO_3$	Sodium Bicarbonate
$NaNO_3$	Sodium Nitrate
$NaH_2PO_4.H_2O$	Monosodium phosphate monohydrate
$FeCl_3.6H_2O$	Ferric chlorid hexahydrate
Na_2.EDTA	Disodium ethylenediamine tetraacetate
$CoCl.6H_2O$	Hexahydrate of anhydrous $CoCl_2$
$MnCl_2.4H_2O$	Manganese(II) Chloride Tetrahydrate
$CuSO_4.5H_2O$	Copper (II) sulfate pentahydrate
$ZnSO_4.7H_2O$	Zinc Sulphate Heptahydrate

Na_2MoO_4	Sodium molybdate dihydrate
ml	Milliliter
^oC/min	Celcius per minute
mL/min	Milliliter per minutes
μd^{-1}	Specific growth rate per day
W	Watt
FID	Flame ionization detector
mm	Millimeter
id	Internal diameter
µm	Micrometer
µL	Microliter
g L^{-1}	Gram per liter

CHAPTER 1

INTRODUCTION

1.1 Background Study

The dietary omega-3 long chain polyunsaturated fatty acids (PUFAs) have shown increase lipid oxidation and prevent high fat and sugar-induced obesity that help in obesity prevention (Pedersen *et al*., 2011). In addition, to achieve the optimum health, contemporary lifestyles must consider about food choices, eating habits and strategies for building up the omega-3 levels in the body. For individuals 'at risk' for diet caused diseases everywhere, even dietary restrictions, prescription remedies, vitamin supplementation, alternative medicine and physical exercise may not be fully protective, preventative or therapeutic without addressing inherent omega-3 fatty acid deficiencies. In other words, the balance of omega fatty acids is important to deliberate. Furthermore, the asserted omega-3: omega-6 ratio has become a model for measuring the proper balance of these fats in oils and the diet. Consequently, omega-3 fatty acids have been focused nowadays in nutrition and medicine especially bioactive lipids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). It has been discovered that certain species of fish and microalgae contain high levels of the essential bioactive omega-3 products EPA and DHA. Meanwhile, the plants also contain diverse levels of omega-3 fatty acids as precursors greatly in the form of α-linolenic acid (ALA). Moreover, there are other dietary long chain fatty acids which are omega-6 fatty acids. Meanwhile, the linoleic acid (LA) is the main omega-6 precursor in plant besides the omega-6 fatty acid arachidonic acid (AA) is bioactive and found in red meat (Doughman *et al.*, 2007).

Accordingly, there are current research suggests increasing accumulated long chain omega-3 fatty acids for health benefits and as natural medicine in several major diseases. Therefore, the first evidence of the important role of dietary intake of omega-3 PUFAs in inflammation was derived from epidemiological observations of the low incidence of autoimmune and inflammatory disorders such as asthma, psoriasis, and type-1 diabetes. The diets containing omega-3 PUFAs as well have also been found to reduce the severity of experimental cerebral and myocardial infarction, to retard autoimmune nephritis and prolong survival of NZB x NZW F_1 mice and also reduce the incidence of breast tumors in rats. Thus, the experimental studies have provided evidence that incorporation of omega-3 fatty acids modifies inflammatory and immune reactions, making omega-3 fatty acids potential therapeutic agents for inflammatory and autoimmune diseases (Simopoulos *et al*., 2002).

Besides that, EPA and DHA from omega-3 fatty acids incorporate into neuronal phospholipids. These omega-3 fatty acids compositions determine the biophysical properties of neuronal membranes and influences neurotransmission. Furthermore, the higher omega-3 PUFAs concentrations lead to higher membrane fluidity, which in turn increases serotonin transport. Dissimilar to *trans*-fats, which have been shown to have negative health consequences, the omega-3 fatty acids are polyunsaturated fatty acids that have been acquainted with many health benefits. In fact they seem to be efficacious in a number of psychiatric and neurological disorders, in particular neurodegenerative diseases. Nevertheless, the omega-3 fatty acids may be helpful in the treatment of dementia and for psychiatric dysfunction in pregnancy and also in breast-feeding (Mazza *et al.,* 2007).

The origin source of omega-3 fatty acids in aquatic ecosystem is microalgae which represent a more direct dietary source of these healthy fatty acids. Besides, there are certain species of microalgae produce high levels of omega-3 fatty acids of EPA and DHA (Doughman *et al*., 2007). The microalgae are able to enhance the nutritional content of conventional food preparations and hence, to positively affect the health of humans and animals. This is regarding to their original chemical composition for example algal lipids that composed of glycerol, from bases esterifies to saturated or unsaturated fatty acids.

Additionally, the microalgae contain many major lipid classes and fatty acids and also constituted as principal producers in the biosphere of some polyunsaturated fatty acids. Besides, the microalgae become current potential sources of these fatty acids and can be provided as a cheap and reliable source to satisfy pharmaceutical requirements (Alonso *et al*., 1998). Whereas, the microalgae are recognized as the primary food source for a large number of aquatic organisms and play a key role in aquaculture development. Therefore, the microalgae also represent a valuable source of nearly all essential vitamins. Otherwise, the microalgae as well as rich in pigments like chlorophyll, carotenoids and phycobiliproteins. Thus, these compositions as represented in Table 1.1 gives microalgae interesting qualities which can be applied in human and animal nutrition (Spolaore *et al*., 2006).

Table 1.1: Particularly interesting microalgal PUFAs

PUFA	Structure	Potential application	Microorganism producer
γ-Linolenic acid (ALA)	18: 3 ω6, 9, 12	Infant formulas for full-term infants Nutritional supplements	*Arthrospira*
Arachidonic acid (AA)	20: 4 ω6, 9, 12, 15	Infant formulas for full-term/preterm infants Nutritional supplements	*Porphyridium*
Eicosapentaenoic acid (EPA)	20: 5 ω3, 6, 9, 12, 15	Nutritional supplements Aquaculture	*Nannochloropsis, Phaeodactylum, Nitzschia*
Docosahexaenoic acid (DHA)	22: 6 ω3, 6, 9, 12, 15, 18	Infant formulas for full-term/preterm infants Nutritional supplements Aquaculture	*Crypthecodinium, Schizochytrium*

Source: Spolaore *et al.,* 2006

In fact, the microalgae are unicellular species that has been acknowledged as a primary source of fatty acids. Moreover, some species of microalgae can be actuated to overproduce typical fatty acids through simple manipulations of the physical and chemical condition of the culture medium. As a result of the profound differences in cellular organization and growth modes and the ability to manipulate their fatty acid content, the microalgae represent a significant source of unusual and valuable lipids and fatty acids (Behrens and Kyle, 2007).

Other than that, the microalgae are present in all persisting earth ecosystems, not just aquatic but also terrestrial, which representing a big variety of species living in a wide range of environmental conditions. The microalgae also can act as a nutritional supplement or represent as a source of natural food colorants because of their diverse chemical properties. Hence, many nutritional and toxicological evaluations have been proved about the suitability of algal biomass as feed supplement. Thus, the genetic improvement of algal strains is also a present challenge nowadays but there is a successful drug discovery which is the most promising aspect of microalgae biotechnology because of the potential is immense (Spolaore *et al*., 2006).

The employment of lipids and fatty acids from microalgae as food components required that the microorganisms to be grown at large scale under controlled growth conditions. For growth of microalgae, there is a few relatively simple condition factors have to be met which are light, carbon source, water, nutrients and a suitably controlled temperature. These variety environmental conditions can be adapted by different microalgae species. Consequently, it is possible to find the best suited species of microalgae for certain local environment conditions or specific growth characteristics (Mata *et al*., 2010).

1.2 Problem Statement

Although the fish actually is one of fatty acid source, but there is a classification by The U.S. Food and Drug Administration about intake of up to 3 grams of fatty acids for daily from fish as GRAS (Generally Regarded as Safe). Intake of fish oil for many months may cause a deficiency of vitamin E, and therefore this constituent is added to many commercial fish oil product. Thus, for regular use of vitamin E-enriched products may lead to the elevated levels of this fat-soluble vitamin. Unfortunately, the fish liver oil already contains the fat-soluble vitamins A and D that may increase the content of vitamins and this will contribute to the toxicity of vitamin in the body and cause to the bone fractures, liver damage or even death.

Moreover, the high doses of fish oil have also been associated with nosebleed and bleeding in the urine because it can decrease platelet aggregation, prolong bleeding time and increase fibrinolysis. Besides, the gastrointestinal upset is common with the use of fish oil supplements. The diarrhea may also occur, with potentially severe diarrhea at very high consumption. There are other side effects also report of increased burping, acid reflux, heartburn, indigestion, abdominal bloating, and abdominal pain.

Potentially harmful contaminants such as dioxins, methyl mercury, and polychlorinated biphenyls (PCBs) are found in some species of fish which have been considered as harmful food contaminants. Therefore, by consuming fish is significantly as the main pathway for human exposure to methyl mercury which is one of the most toxic forms of mercury that has been reached relatively high concentrations in most species of fish (Gochfeld and Burger, 2005). Meanwhile, it has been proved that some fish oil capsules sold as health supplements for providing the fatty acids content have illegally undisclosed unnecessarily high levels of contamination with polychlorinated biphenyls (PCBs) compounds. PCBs and methyl mercury are believed to have long half-lives in the body and can accumulate in people who consume fish on a frequent basis. The recommendation currently suggest by limiting intake of fish in dietary. For the case of PCBs, it is suggested that consumers to reduce their exposure to these contaminants by

removing the fat from the fish before cooking them, because it is distributed throughout skin, muscle and organs of the fish.

Besides, the global warming issue which is one of the hottest global issues because of the big impact on our universe and environment. The global warming affects an increase in the average temperature of the earth's atmosphere, especially a sustained increase sufficient to cause climatic change. Therefore, carbon dioxide (CO_2) actually is the main greenhouse gas which contributes about 75% of Green House Gases (GHGs) compositions (Purba and Taharuddin, 2010). There are many attempts to recover CO_2 from atmosphere including physical and chemical treatments have been used. The reduction of CO_2 using microalgae as CO_2 fixers can be used as an efficient solution in biological approach (Sheng-Yi *et al.*, 2008). The microalgae contain organic substances such as polysaccharides, lipid, vitamins, minerals, and other bioactive substances. Hence, the photosynthetic CO_2 fixation by microalgae which have higher photosynthetic efficiency is the most effective carbon sequestration method on earth and have been thought to be a feasible technology with energy-saving and environment-friendly (Tang *et al*., 2011).

There is an additional problem by using fish as a source of fatty acid which affects the environmental issue because of carbon dioxide emissions associated due to over-fishing. In addition, over-fishing practices and depletion of some stocks may become so severe that may be regarded as economically extinct happened. Some overfishing practices may deplete populations and unsustainable at ecosystem level (Coll *et al.*, 2008). Hence, the microalgae are used as the new alternative source of fatty acid besides become as a prevention of marine pollution (Venegas-Calerón *et al*., 2010). Consequently, microalgae oil has potential benefits compared to fish oil which are high level content of fatty acids, has lower risk of contamination with heavy metals and more natural taste instead of fishy aftertaste.

1.3 Objectives

To study the effects on carbon dioxide and light intensity in producing high yield of omega-3 fatty acid from the strain of *Nannochloropsis* sp.

1.4 Scope study

To analyze the effect of carbon dioxide concentration in air stream on lipid accumulation of *Nannochloropsis* sp. in order to produce the high quantity of omega-3 fatty acid. The ranges of carbon dioxide percentage used are 2%, 5%, 10% and 15%.

To identify the light intensity required for supporting the carbon fixation through the photosynthesis process of *Nannochloropsis sp.* in maximizing the production of omega-3 fatty acid. There are ranges of irradiance levels which are 31 µmol photons m^{-2} s^{-1}, 82 µmol photons m^{-2} s^{-1} , 125 µmol photons m^{-2} s^{-1} and 156 µmol photons m^{-2} s^{-1} .

1.5 Rationale and Significance

The rationale of this case study is providing the empirical evidence that microalgae have high potential in production of fatty acids. Microalgae have level of oil about 20%–50% (Huang *et al.*, 2010). Production of fatty acid from microalgae has been advanced only in the last decade and has the advantages of lacking unpleasant fish odor, reduced risk of chemical contamination and better purification potential. Therefore, there is the fatty acids from microalgae provide a sustainable and non-contaminated source of these important fatty acids for human nutrition. Thus, not only do these fatty acids help to improve chronic and acute human diseases for examples cardiovascular disease, obesity, type-2 diabetes and metabolic syndrome thus consequently reducing public expenditure on the healthcare system, but also contribute to correct development of neonates and infants (Venegas-Calerón *et al*., 2010).

Additionally, the increasing concentration of greenhouse gases as global issue in the atmosphere has received great concern to world population regarding the matter of global warming. The carbon dioxide (CO_2) which is the principal greenhouse gas, account for 76.7% (v/v), and its concentration have increased rapidly since the onset of industrialization. The anthropogenic emission of CO_2 from coal-fired thermoelectric plants is responsible for up to 7% (v/v) of global CO_2 emissions, meanwhile about 10% to 15% (v/v) of the flue gases emitted from the power plants being in the form of carbon dioxide gas. Thus, microalgae as the photosynthetic microorganisms use inorganic carbon for growth and hence can convert CO_2 from a point source into biomass. CO_2 biofixation method using microalgae is the most important and the most effective carbon sequestration method on earth because of the photosynthetic efficiency, higher biomass production and also faster growth compared to other energy crops. In the applications of microalgae for fixation of carbon dioxide, the tolerance to the CO_2 biomass and fatty acid content of the microalgae are of great important outcome (Tang *et al*., 2011).

Moreover, regarding to the over-fishing activity and concerns about pollution of the marine environment demonstrate a need to develop alternative and sustainable sources of omega-3 fatty acids. Consequently, there is a number of different strategies have been considered, using aquatic organisms as other source of fatty acids. Therefore, regarding to the matter about the sustainability of global fish stocks which actually the main sources of fatty acids because marine fish stocks are in severe decrease as a result of decades of over-fishing. Moreover, environmental pollution of marine ecosystems has resulted in the accumulation of dioxins, content of heavy metals and polychlorinated biphenyls in fish that to be doubtful about the benefits of fish consumption in dietary that will give impact to the human health (Venegas-Calerón *et al*., 2010).

CHAPTER 2

LITERATURE REVIEW

2.1 Fatty Acids

Lipids consist of numerous fat-like chemical compounds that are insoluble in water but soluble in organic solvents. The lipid compounds include monoglycerides, diglycerides, triglycerides, phosphatides, cerebrosides, sterols, terpenes, fatty alcohols, and fatty acids. In addition, lipids are the sole sources of polyunsaturated fatty acids (PUFAs) in microalgae. Besides, the lipid contents significantly are influenced by environmental conditions and can be physiologically manipulated, which results in variation of fatty acid content in the lipid pool (Chen *et al*., 2007). In most cases, lipids are not static but will change actively during normal metabolism or in response to cellular stimuli. The lipids turn over and are biosynthetically remodeled during the cell cycle or experience chemical restructuring to new lipid species with altered properties that serve as bioactive mediators in various signaling pathways. The rapid dynamic change will add to the complexity of lipid metabolism. Furthermore, as an integral building block of semi-permeable membranes, lipids not only function to form barriers between cells and extracellular space and between intracellular organelle compartments but they also affect directly the physical and functional properties of cell membranes (Quehenberger *et al*., 2009).

Concurrently, the fatty acids are merely carboxylic acids with long hydrocarbon chains. The hydrocarbon chain length may vary from 10 to 30 carbons. The non-polar hydrocarbon alkane chain is an important counter balance to the polar acid functional

group. Additionally, the fatty acids also consist of the elements carbon (C), hydrogen (H) and oxygen (O) arranged as a carbon chain skeleton with a carboxyl group (-COOH) at one end. The saturated fatty acids (SFAs) have all the hydrogen that the carbon atoms can hold and have no double bonds between the carbons. Moreover, monounsaturated fatty acids (MUFAs) have only one double bond besides polyunsaturated fatty acids (PUFAs) have more than one of double bond. For nomenclature of fatty acids, it begins with the number of carbons, then after a colon, the number of double bonds, followed by the position of the first double bond counting from the omega position in carbon chain. Majority of saturated fatty acids in plasma are palmitic acid (C16:0) and stearic acid (C18:0). Meanwhile, the major monounsaturated fatty acid is oleic acid (C18:1n9), which contains only one double bond. Other than that, there are oleic, stearic and palmitic acids were found in the hydrolysate of the lipid component, which in average about 1.5 % of the lipopolysaccharide (LPS) from the cell wall of microalgae (Mikheyskaya *et al*., 1976).

Nevertheless, it has been found that many of the effects of fatty acids on immune and inflammatory responses are not dependent on eicosanoid generation. Furthermore, the fatty acids have also been found to modulate the process of phagocytosis, reactive oxygen species production, cytokine production, leukocyte migration and also for interfering with antigen presentation by macrophages. The importance of fatty acids in immune function has been corroborated by many clinical trials in which patients which have been showed improvement when submitted to fatty acid supplementation. Several mechanisms have been proposed to explain fatty acid modulation of immune response, such as changes in membrane fluidity and signal transduction pathways, regulation of gene transcription, protein acylation, and also calcium release (Pompeia *et al.,* 2000).

In addition, the fatty acids are important components of other intracellular communication molecules, for examples platelet activating factor, diacylglycerol and ceramides. Despite, even the least sophisticated fatty acids such as the volatile fatty acids or the long-chain saturated fatty acids have important roles in cell metabolism, structure and regulation, with considerable implications in the immune function when the cells in question are leukocytes. Furthermore, the immune system works with specific and

nonspecific recognition of foreign molecules, leading to their inactivation or destruction also by specific or nonspecific means. In spite of that, the fatty acids fulfill a variety of roles within immune cells that function as fuels for generation of energy, components of cell membrane phospholipids contributing to the physical and functional properties of those membranes, covalent modifiers of protein structure that affect the cellular, location and function of proteins, regulators of gene expression either through effects on receptor activity, on intracellular signaling processes, or on transcription factor activation and precursors for synthesis of bioactive lipid mediators like prostaglandins (PGs), leukotrienes (LTs), lipoxins and resolvins (Calder, 2008).

There are many types of fatty acids which are divided into two series of essential fatty acids, one has a double bond three carbon atoms removed from the methyl end and the other has a double bond six carbon atoms removed from the methyl end. Several types of fatty acids which are arachidic acid, stearic acid, palmitic acid, erucic acid, oleic acid, arachidonic acid, linoleic acid and linoleic acid as illustrated in Figure 2.1.

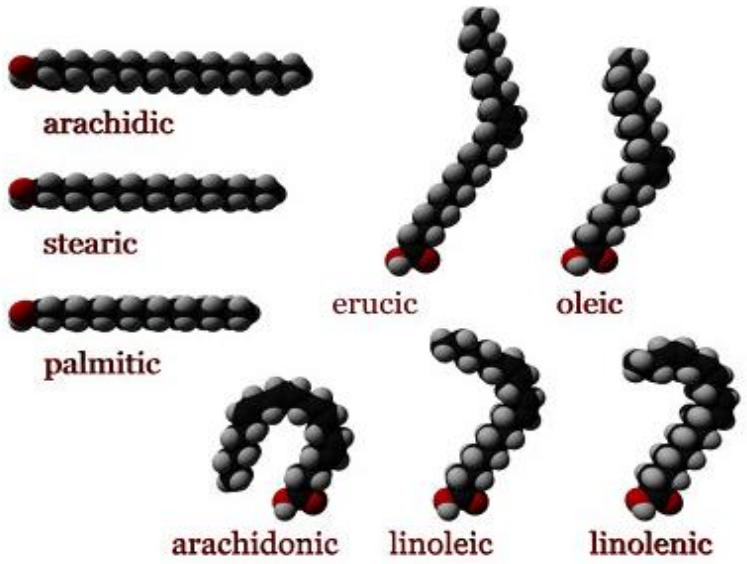

Figure 2.1: Dimensional representations of several fatty acids

Source: biochemistryquestions.wordpress (2008)

Table 2.1: Chemical Names and Descriptions of some Common Fatty Acids

Common Name	Chain Length	Double Bonds	Scientific Name
Arachidic acid	C20	0	eicosanoic acid
Stearic acid	C18	0	octadecanoic acid
Palmitic acid	C16	0	hexadecanoic acid
Myristic acid	C14	0	tetradecanoic acid
Lauric acid	C12	0	dodecanoic acid
Palmitoleic acid	C16	1	9-hexadecenoic acid
Palmitoleic acid	C16	1	*cis*-9-Hexadecenoic acid
Oleic Acid	C18	1	9-octadecenoic acid
Linoleic Acid	C18	2	9,12-octadecadienoic acid
Alpha-Linolenic Acid	C18	3	9,12,15-octadecatrienoic acid
Arachidonic Acid	C20	4	5,8,11,14-eicosatetraenoic acid
Eicosapentaenoic acid	C20	5	5,8,11,14,17-eicosapentaenoic acid
Behenic acid	C22	0	docosanoic acid
Erucic acid	C22	1	13-docosenoic acid
Docosahexaenoic acid	C22	6	cervonic acid

Source: Schroeder and Soler-Argilaga, 1997

Besides, most naturally occurring fatty acids have a chain of an even number of carbon atoms, from 4 to 28. As shown in Table 2.1, the fatty acids are frequently represented by a notation such as C18 that indicates that the fatty acid consists of an 18-carbon chain.

2.1.1 Omega-3 Fatty Acid

Omega-3 fatty acids are important nutrients that are involved in many bodily processes. There are three fatty acids compose in the omega-3 family which are α-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid. The omega-3 fatty acids make up a family of essential fats that humans are unable to synthesize *de novo* and need to be taken in dietary. The primary *de novo* synthesis sources of very long chain PUFAs are marine microbes for example algae which form the base of an aquatic food web that culminates in the accumulation of these fatty acids in the lipids of the fish. Besides, PUFAs consist of 20 carbons or more fatty acids in length with three or more methylene-interrupted double bonds in the *cis* position. Moreover, these fatty acids can be classified into two main families which are omega-6 and omega-3 families, deriving on the position of the first double bond proximal to the methyl end of the fatty acid. PUFAs are also known as vital constituents of human metabolism. In particular, there is plentiful evidence for the health-beneficial properties to humans of dietary consumption of omega-3 PUFAs for example eicosapentaenoic acid (EPA;20:5Δ5,8,11,14,17) and docosahexaenoic acid (DHA;22:6 Δ4,7,10,13,16,19). This dietary requirement is almost certainly due to the fact that humans have limitation of capacity to synthesize these fatty acids from the essential precursor α-linolenic acid (ALA;18:3Δ9,12,15), therefore dietary intake of these fatty acids is a key aspect of human nutrition (Venegas-Calerón *et al.*, 2010).

According to Surette (2008), omega-3 fatty acids are being increasingly promoted as crucial dietary components for health and disease prevention. In addition, there is an increasing number of foods that are not traditional sources of omega-3 fatty acids, such as dairy and bakery products are now being fortified with small amounts of these fatty acids. This recent promotion of omega-3 fatty acids has likely been driven by recommendations for omega-3 fatty acid consumption made by scientific groups such as the American Heart Association. Whence, the research for the molecular and cellular mechanisms by which omega-3 fatty acids affect health and disease has led to a large body of evidence which suggests that these dietary lipids modulate numerous processes, including brain and visual development, inflammatory reactions, thrombosis and carcinogenesis. Simultaneously, the

omega-3 fatty acids have many needs in the body, including ß-oxidation for energy, storage in depot fat or incorporation into phospholipids, which form the major structural components of all cellular membranes. Consequently, not all dietary fatty acids are created equally, because humans do not have the enzymatic machinery required to synthesize omega-3 fatty acids, hence, they must be obtained from the diet. Even among dietary polyunsaturated fatty acids, there are different families of compounds, and this is at the heart of the difference between omega-3 fatty acids and other dietary lipids.

Furthermore, omega-3 fatty acids from fish are not to be confused with those from plant sources for examples flax and canola oil. These plant oils are enriched in an omega-3 fatty acid called α-linolenic acid, which is a metabolic precursor of the omega-3 fatty acids found in fish and fish oils as shown in Figure 2.2. Even though we are adept to convert dietary α-linolenic acid into eicosapentaenoic, docosapentaenoic and docosahexaenoic acids which are found in fish and fish oils, this conversion is not effective and cannot be applied for people who consume a typical Western diet. Consequently, following the utilization of foods containing α-linolenic acid, our tissues are exposed to very little of the types of omega-3 fatty acids found in fish and fish oils. Some beneficial biological activity has been attributed to plant-derived omega-3 fatty acids; however, the associated health benefits are likely independent of the conversion of α-linolenic acid to the fatty acids found in fish.

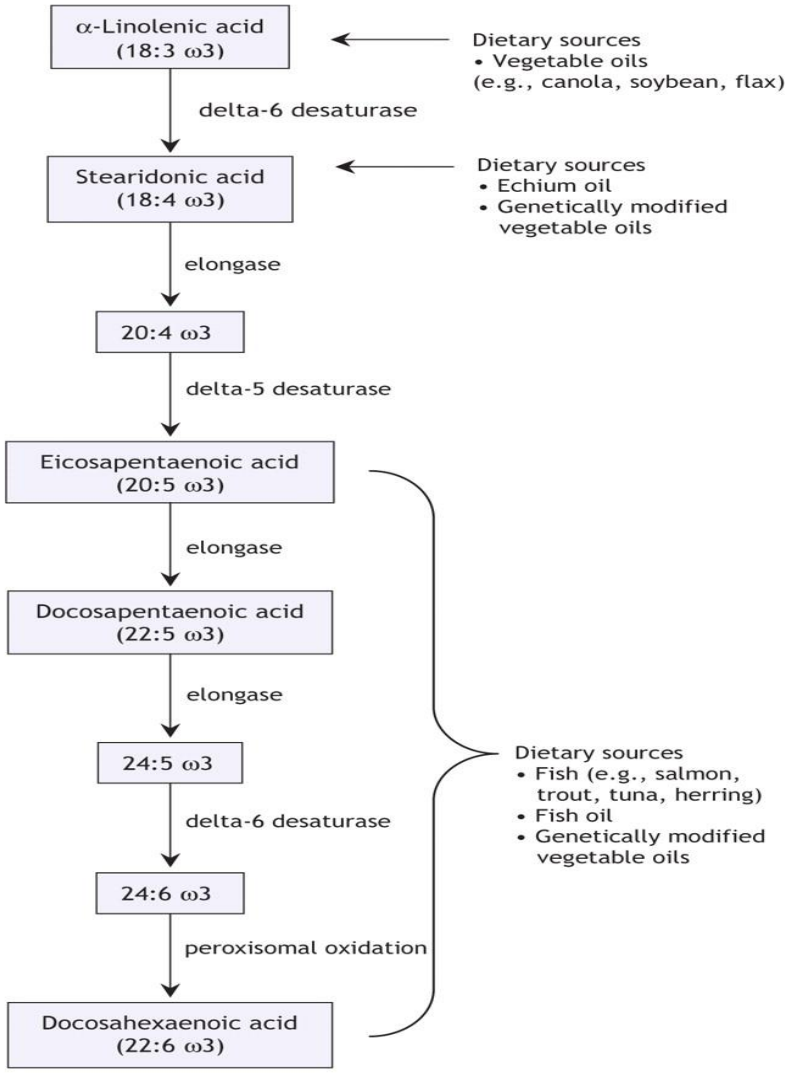

Figure 2.2: Metabolism and dietary sources of the omega-3 family of polyunsaturated fatty acids

Source: Surette., 2008

2.1.1.1 Eicosapentaenoic acid

Eicosapentaenoic acid (EPA, 5,8,11,14,17-*cis*-eicosapentaenoic acid)**,** is an omega-3 C_{20}-polyunsaturated fatty acid that is metabolically active has been shown to have several highly beneficial effects such as preventing atherosclerosis and alleviating inflammatory conditions. Accordingly, EPA and its derivatives have proved beneficial in prevention and treatment of certain medical conditions comprising coronary heart disease, blood platelet aggregation, abnormal cholesterol levels and several carcinomas. EPA is effectual also in arresting and reducing the tumor growth (Belarbi *et al*., 2000). Moreover, EPA also plays an important role in human health in the prevention of cardiovascular diseases and to inhibit tumor inflammation have been reported (Hoshida *et al*., 2005).

The conventional and commercial source of EPA is marine fish oil, but higher amount of EPA can be produced by the use of algae. In addition, there are important concerns regarding contamination of fish oil with pesticides and heavy metals besides become a rather unsatisfactory source because of problems of, taste, odor and stability. Additionally, the presence of considerable amounts of other PUFAs in the fish oil perplexes the EPA purification process, resulting in high retail prices of the pure product. These causes have led for discovery of alternative EPA sources. Several microorganisms have long been acknowledged as potential EPA producers. Fungi, especially of the order *Mucorales*, and bacteria of the genera such as *Shewanella*, *Alteromonas*, *Flexibacter* and *Vibrio* can accumulate relatively large amounts of EPA and have been indicated as possible sources of this long chain fatty acid. However, the attributes of bacterial and fungal fermentations to compete economically with traditional sources of omega-3 fatty acids is limited by low productivities and excessively long fermentation times. Species of marine microalgae, such as *Porphyridium cruentum*, *Phaeodactylum tricornutum*, *Isochrysis galbana* and the eustigmatophytes of *Nannochloropsis oculata*, *Nannochloropsis* sp. and *Monodus subterraneus* have also been proposed for commercial production of EPA under autotrophic conditions (Zitteli *et al*., 1998).

2.2 Microalgae

Recent research has studied the importance of fatty acids in health benefits including for skin, respiratory system, circulatory system, brain and heart disease by lowering cholesterol levels. Many people consume fatty acids as their nutritional supplements in the form of fish oil or olive oil in order to obtain these health benefits. There are some concerns identified by taking fish oil supplements which are the cold water fish concentrate environmental pollutants in their bodies, and may contain harmful toxic amounts of heavy metals, pesticides or dioxin (Gochfeld and Burger, 2005). Therefore, the fish oil supplementation can cause unpleasant side effects, including flatulence, diarrhea and unpleasant breath. Besides that, the fish oil manufacturing puts pressure on the already threatened cold water fish populations (Coll *et al.*, 2008).

Currently, the source of fatty acids is fish oil. Thus, it is a need to find another alternative in order to get the healthy and free contaminant of fatty acids. Hence, many discoveries of fatty acids sources have been found for example the aquatic microorganisms which are microalgae. Despite of that, while fish oil is already widely known for having an unusually high amount of fatty acids, these fish do not actually produce the fatty acids by themselves. This because the fish actually extracts the fatty acids from the microalgae, thus the microalgae is absolutely the primary source of these fatty acids (Behrens and Kyle, 2007). Microalgae grow in the marine environments as well as freshwater. Microalgae are also friendly environmental and sustainable source of total fatty acids compared to the fish oil. Strain of microalgae for examples *Spirulina* sp. and *Nannochloropsis* sp. can produce long chain of fatty acids that are commonly not present in higher plants (Purba and Taharuddin, 2010). Besides, the fatty acids extracted from algae can be used in many applications for examples in fortified foods, or the biomass can be used directly as a feed additive in various animal industries such as aquaculture or poultry. Many microalgae species showed that, they can be induced to accumulate substantial quantities of lipids, thus contributing to a high oil yield.

Besides that, the average lipid content varies between 1% - 70% but under certain conditions some species can produce 90% of dry weight (Mata *et al.,* 2010). The microalgae have many advantages which are the fatty acids constitutions similar to common vegetables oil, obtain as high as 85% of the dry weight under the certain condition, have short time of growth cycle and the composition is relative single in microalgae (Huang *et al*., 2010). The microalgae also can be grown on low to no-cost nutrients, which make them an economically viable source of fatty acids. Microalgae have many applications because it can provide feedstock for several different types of renewable fuels such as biodiesel, methane, hydrogen, ethanol, among others. The algae biodiesel produced contains no sulfur and performs as well as petroleum diesel, while reducing the emissions of particulate matter, carbon monoxide, and hydrocarbons. Other than that, microalgae contribute in removal of carbon dioxide from industrial fuel gases by algae biofixation method and also reducing the green house gases emissions of a company or process. Besides, the microalgae undergo the wastewater treatment by removal of NH^{4+}, NO_3^-, PO_4^{3-} by making the microalgae to grow using these water contaminants as growth nutrients.

Moreover, after the oil extraction process, the resulting algae biomass can be processed into ethanol, methane, livestock feed, used as organic fertilizer due to its high nitrogen and phosphorus ratio, or simply burned for energy cogeneration for examples electricity and heat. Moreover, there are valuable applications in different industrial sectors, including a large range of fine chemicals and bulk products, such as fats, polyunsaturated fatty acids, oil, natural dyes, sugars, pigments, antioxidants, high-value bioactive compounds, and other fine chemicals and biomass. Last but not least, because of this variety of high-value biological derivatives, with many possible commercial applications, the microalgae can revolutionize a large number of biotechnology field including biofuels, cosmetics, pharmaceuticals, nutrition and food additives, aquaculture and also pollution prevention (Mata *et al.*, 2010).

2.2.1 Strain of *Nannochloropsis* sp.

According to Mata *et al.* (2010), many microalgae species can be induced to accumulate substantial quantities of lipids thus contributing to production of high oil. Table 2.2 presents both lipid content and lipid and biomass productivities of different marine and freshwater microalgae species, showing significant differences between the various species. The average lipid content varies between 1% and 70% but, it also depend to the cell growth under certain conditions because some species can reach about 90% of dry weight. The strain of *Nannochloropsis* sp. is an interesting microalga in the field of marine biotechnology because of its high content of lipid in the range for dry weight biomass about 12% to 53%. Furthermore, it has been analyzed that the fatty acid compositions of seven fresh water microalgae species showing that all of them synthesized the fatty acids of C14:0, C16:0, C18:1, C18:2 and C18:3. There is also the relative intensity of C16:4 and C18:4 in *Ankistrodesmus* sp., C18:4 and C22:6 in *Isochrysis* sp., C16:2, C16:3 and C20:5 in *Nannochloris* sp., C16:2, C16:3, and C20:5 in *Nitzschia* sp. Furthermore, the strain of *Nannochloropsis* sp. has a high lipid content about 46%, determined with the extraction process of Bligh–Dyer method, by using methanol–chloroform mixture (2/1, v/v) as the extraction solvent), and is considered to be a promising green microalgae for fuel products (Pan *et al*., 2010).

Nannochloropsis sp. is small, nonmotile spheres which do not express any distinct morphological features, and cannot be distinguished by either light or electron microscopy. The ultrahigh cell density cultures of *Nannochloropsis* sp., a unicellular marine widely used in mariculture as a very good source of omega-3 polyunsaturated fatty acids. The physiology of biosynthesis of lipids and polyunsaturated fatty acids, with particular reference to EPA, has been thoroughly investigated in this alga (Zou *et al.*, 2000). The strain of *Nannochloropsis* sp. is established as a marine eustigmatophyte currently cultivated in many aquaculture hatcheries as the basis of an artificial food chain. Moreover, because of its high amount of EPA content, this eustigmatophyte is considered as a good potential for particularly EPA source (Zou and Richmond, 1999).

Table 2.2: Lipid content and productivities of different microalgae species

Microalgae species	**Lipid content (% dry weight biomass)**	**Lipid productivity (mg/L/day)**	**Volumetric productivity of biomass (g/L/day)**	**Areal productivity of biomass (g/m^2/day)**
Ankistrodesmus sp.	24.0 -31.0	-	-	11.5-17.4
Botryococcus braunii	25.0 -75.0	-	0.02	3.0
Chaetoceros muelleri	33.6	21.8	0.07	-
Chaetoceros calcitrans	14.6-16.4/39.8	17.6	0.04	-
Chlorella vulgaris	5.0 – 58.0	11.2 – 40.0	0.02 – 0.20	0.57 – 0.95
Chlorella sp.	10.0 – 48.0	42.1	0.02 – 2.5	1.61 – 16.47/25
Chlorococcum sp.	19.3	53.7	0.28	-
Crypthecodinium cohnii	20.0 – 51.1	-	10	-
Dunaliella salina	6.0 – 25.0	116.0	0.22 – 0.34	1.6 – 3.5/20-38
Dunaliella sp.	17.5 – 67.0	33.5	-	-
Ellipsoidion sp.	27.4	47.3	0.17	-
Euglena gracilis	14.0 – 20.0	-	7.70	-
Haematococcus pluvialis	25.0	-	0.05 – 0.06	10.2 – 36.4
Isochrysis galbana	7.0 – 40.0	-	0.32 – 1.60	-
Isochrysis sp.	7.1 – 33.0	37.8	0.08 – 0.17	-
Monodus subterraneus	16.0	30.4	0.19	-

Microalgae species	Lipid content (% dry weight biomass)	Lipid productivity (mg/L/day)	Volumetric productivity of biomass (g/L/day)	Areal productivity of biomass ($g/m^2/day$)
Monallanthus salina	20.0 – 22.0	-	0.08	12
Nannochlorosis sp.	20.0 – 56.0	60.9 – 76.5	0.17 – 0.51	-
Nannochloropsis oculata	22.7 – 29.7	84.0 – 142.0	0.37 – 0.48	-
Nannochloropsis sp.	12.0 – 53.0	37.6 – 90.0	0.17 – 1.43	1.9 – 5.3
Neochlorosis oleoabundans	29.0 – 65.0	90.0 – 134.0	-	-
Nitzschia sp.	16.0 – 47.0	-	-	8.8 – 21.6
Oocystis pusilla	10.5	-	-	40.6 – 45.8
Pavlova salina	30.9	49.4	0.16	-
Pavlova lutheri	35.5	40.2	0.14	-
Phaeodactylum tricornutum	18.0 – 57.0	44.8	0.003 – 1.9	2.4 – 21
Porphyridium cruentum	9.0 – 18.8/ 60.7	34.8	0.36 – 1.50	25
Scenedesmus obliquus	11.0 – 55.0	-	0.004 – 0.74	-
Scenedesmus quadricauda	1.9 – 18.4	35.1	0.19	-
Scenedesmus sp.	19.6 – 21.1	40.8 – 53.9	0.03 – 0.26	2.43 – 13.52
Skeletonema sp.	13.3 – 31.8	27.3	0.09	-

Source: Mata *et al*., 2010

2.3 Carbon Dioxide

The global warming by carbon dioxide (CO_2) emission, mainly from the combustion of fossil fuel, has been estimated to be 2×10^{10} tons/year. This phenomenon is caused by incremetation of atmospheric CO_2 level that besides other greenhouse gases, such as methane and chlorofluorocarbons. The operation in power plants that burn enormous amounts of fossil fuels, for examples coal and oil for the generation of steam and the CO_2 from these plants accounts for more than 16% of total CO_2 emissions. Thus, there are possible engineering improvements and policy decisions have been widely discussed in attempting to decrease the rate of CO_2 level in the atmosphere. The elimination process of CO_2 is possible done by physicochemical methods for examples wet absorption, dry adsorption and membrane separation techniques but for further disposal of the trapped CO_2 become costly process. Consequently, the exhaust gas from power plants could be a useful source of CO_2 for the mass culture of microalgae that used as requirement. Moreover, the microalgae fixedis incorporated into carbohydrates, lipids, proteins, energy, chemicals, foods which can be produced as algal biomass. The processes leading to the conversion of algal biomass to such useful products would be an economical and environmental method for CO_2 fixation and disposal, because they would indirectly decrease dependence on fossil fuels, which are not renewable (Negoro *et al.,* 1991).

Hence, there is a bioregenerative methods using photosynthesis process by microalgae cells have been applied in order to reduce the atmospheric of CO_2 besides contribute in reducing the greenhouse gases which accumulating dramatically in Earth's atmosphere as a result of human activities and industrialization. The increasing concentration of greenhouse gases causes absolutely critical global warming that affect to increase the temperatures of the surface air and subsurface ocean. Thus, this biogenerative method can make sure a safe and reliable living environment. Furthermore, the marine microalgae are expected as a suitable candidate due to the high capability for metabolite exchange in the photosynthesis process and easily cultured in sea water which solubilizes the high amount of CO_2 (Chiu *et al.,* 2009).

2.4 Light Intensity

The photosynthesis process comprising two steps of reactions which are light reactions that only occur when the cells are illuminated, and the other one is carbon-fixation reactions, also known as dark reactions, that occur both in the presence and absence of light. Thus, for the first step the cells transform the light energy into the chemical energy, which then the chemical energy is stored in high-energy compounds for later use in the carbon-fixation reactions. Moreover, the use of these photosynthetic pathways in environmental engineering processes requires the use of solar energy besides in development of clean and technology processes. Hence, the cells use the light energy by way of exergonic reactions, producing energy that is used in the synthesis of compounds as from carbon dioxide fixation by way of endergonic reactions. However, there is one of the operational problems of this type of technology refers to the lack of availability of light energy for whole time periods. The light regimes to which the cultures are submitted are considered to be an important factor in the productivity and yield of photosynthetic reactions (Jacob- Lopes *et al.*, 2009).

CHAPTER 3

METHODOLOGY

3.1 Material

3.1.1 Strain

The microalgae used are the strain of *Nannochoropsis* sp.

3.1.2 Medium

The growth medium for culturing strain of *Nannochloropsis* sp. is modified f/2 medium in artificial sea water which consists of stock solution and trace elemental solution. The stock solution has the following composition (per liter): 29.23 g NaCl, 1.105 g KCl, 11.09 g $MgSO_4.7H_2O$, 1.21 g tris-base, 1.83 g $CaCl_2.2H_2O$, 0.25 g $NaHCO_3$ and 3.0 mL of trace elemental solution. The trace elemental solution (per liter) includes 75 g $NaNO_3$, 5 g $NaH_2PO_4.H_2O$, 4.36 g Na_2.EDTA, 3.16 g $FeCl_3.6H_2O$, 180 mg $MnCl_2.4H_2O$, 10 mg $CoCl.6H_2O$, 10 mg $CuSO_4.5H_2O$, 23 mg $ZnSO_4.7H_2O$, 6 mg Na_2MoO_4, 100 mg vitamin B_1, 0.5 mg vitamin B_{12} and 0.5 mg Biotin.

3.2 Equipment

3.2.1 Photobioreactor

The photobioreactor in this experiment is used for cultivating microalgae cell of *Nannochloropsis* sp. on purpose to fix the parameter effect of carbon dioxide and light intensity. It has been provided white fluorescent lamp with four different level of light intensity and aerated with mixture of air and various flow rate of carbon dioxide.

3.2.2 Autoclave

This equipment is used to sterilize the glassware apparatus and materials by subjecting with high pressure saturated steam at 121 °C for 20 minutes.

3.2.3 Oven

This equipment is used in determination of dry weight for cell biomass of *Nannochloropsis* sp. at 24 hours of period. The moisture of sample is evaporated by oven drying and the total dry matter is determined gravimetrically as residue remaining after drying.

3.2.4 Ultrasonic

This equipment is applied in sonoporation process for disruption of *Nannochloropsis* sp. cell membranes and to release the cellular contents.

3.2.5 Centrifuge

This equipment is used for harvesting the algal cells. Centrifugation involves the application of centripetal acceleration to separate the algal growth medium into regions of

greater and less densities. Once separated, the algae can be removed from the culture by simply draining the excess medium.

3.2.6 Incubator shaker

The equipment used to provide uniform agitation continuously at room temperature for 10 minutes in allowing the organic and aqueous layer of *Nannochloropsis* sp. with methanol/chloroform solution to separate.

3.2.7 Gas Chromatography

This equipment is used for analytical method by identifying the omega-3 fatty acids compounds present in the cell sample of *Nannochloropsis* sp. and also to determine the purity of sample.

3.3 Experimental Procedure

3.3.1 Microalgae cultures in experimental system with photobioreactor

Strain of *Nannochloropsis* sp. is obtained from the microalgae collection was cultured in an Erlenmeyer flask with 300 mL working volume of modified f/2 medium under 26 ± 1 ºC and provided with different light intensity of 31 µmol photons m^{-2} s^{-1}, 82 µmol photons m^{-2} s^{-1}, 125 µmol photons m^{-2} s^{-1} and 156 µmol photons m^{-2} s^{-1}. Gas provided as different concentration of CO_2 mixed with ambient air that prepared with a volumetric percentage of CO_2 and filtered by 0.45 µm of PTFE filter to give CO_2 concentrations of 2%, 5%, 10% and 15%. The microalgae cultures were aerated continuously with gas provided via bubbling from the top of flask by tubing with an aeration rate of 200 mL/min. Different concentrations of CO_2 aeration were mixed with air and pure CO_2 and adjusted by gas flow meter. After 7 days of cultivation, the microalgae cells harvested by centrifugation

at 3000xg for 5 minutes were resuspended with 50 mL of fresh medium and separated for further experiments (Chiu *et al*., 2009).

3.3.2 Microalgae cell growth and dry weight

Meanwhile, the specific growth rates (μd^{-1}) were calculated till the end of cultivation everyday by applying the analysis of dry weight per 10 ml of cell culture according to the equation as follows:

$$\text{Specific Growth Rate } (\mu d^{-1}) = \frac{\ln (W_f / W_0)}{\Delta \text{ Time}} \qquad \textbf{(Equation 3.1)}$$

W_f = Final Biomass Concentration

W_0= Initial Biomass Concentration

The cell cultures were sampled at 24 hours interval. The cells were filtered by vacuum pump. Then, the membranes filter used need to be dried at 105 °C for 16 hours to get the dried weight analysis (Chiu *et al*., 2009).

3.3.3 Extraction of total lipids

The dried algal sample extracted in triplicate for 20 minutes with 20 ml of chloroform/methanol (2:1 v/v) at 25 ºC in a 100 W sonication bath. After sonication, 2 ml of distilled water was added to each extract and the contents were mixed by vortexing for 30 seconds. Tubes containing samples were then incubated for 10 minutes at room temperature to allow the organic and aqueous layers to separate. After removing and saving the bottom or known as organic layer, the aqueous layer was re-extracted by adding 6.6 ml chloroform, mixed by vortexing for three pulses of 10 seconds each. The resulting extracts were stored at 4 ºC prior to remove the aliquots for oil and fatty acid analysis (Mulbry *et al.*, 2009).

3.3.4 Measurement of eicosapentaenoic acid content by gas chromatography

For determination of eicosapentaenoic acid content, it can be carried out by using Gas Chromatograph-FID equipped with a DB-WAX 123-7032 (0.32 mm × 30 m id x 0.25 μm film thickness Agilent 6890). The hydrogen is used as carrier gas at 1.0 ml/min. The samples were injected at 1μL volume at the following condition with the column temperature was 40ºC during the first 3 minutes, then ramped to 180ºC with 40 ºC/min and was maintained for 3 minutes, finally, ramped up at 10 ºC/min to 270 ºC, and maintained for 5 minutes. Meanwhile, for injector and flame ionization detector temperature was 250ºC and 250 ºC respectively. The pure standard used is eicosapentaenoic acid.

CHAPTER 4

RESULT AND DISCUSSION

4.1 The Growth and Biomass Concentration of *Nannochloropsis* sp.

4.1.1 Effect of Carbon Dioxide on Growth and Biomass Concentration of *Nannochloropsis* sp.

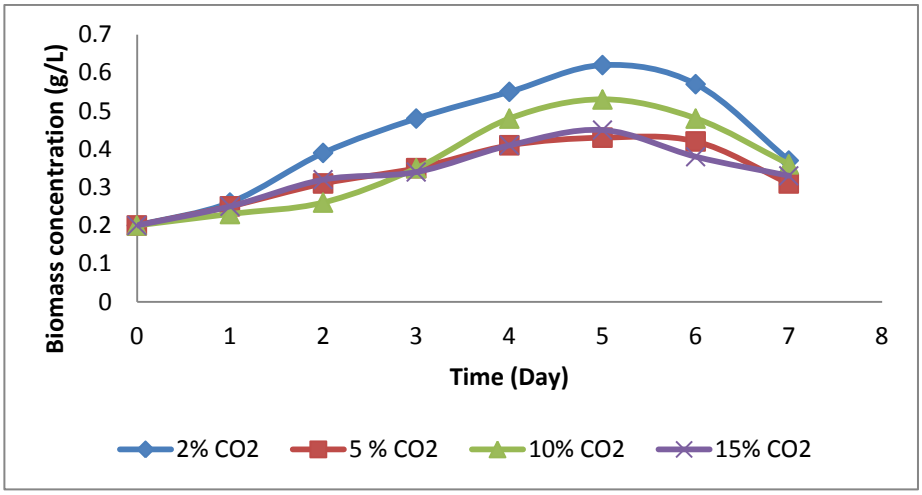

Figure 4.1: Effect of the CO_2 concentration on the growth of *Nannochloropsis* sp.

The effect of the concentration in airstream on the growth of *Nannochloropsis* sp. was studied in a batch culture incubated at 26 ± 1 ºC and 156 µmol photons m^{-2} s^{-1}. The initial biomass inoculums was 0.0020 g L^{-1} and the cultures were aerated with mixture of air and different concentration of CO_2 which are 2%, 5%, 10%, and 15%. The specific

growth rate was calculated for each sample in the experiment. Figure 4.1 shows the microalgal growth aerated with different CO_2 concentrations. At fifth day of cultivation, the growth of 2% CO_2 aerated cultures reached a plateau stage at 0.024 μD^{-1} and the biomass concentration of *Nannochloropsis* sp. was 0.6200 g L^{-1}. Meanwhile, the growth of microalgae aerated with 5%, 10%, and 15% CO_2 are 0.0095 μD^{-1}, 0.0198 μD^{-1} and 0.0186 μD^{-1} , respectively. The sample cultured with aeration 2% of CO_2 showed an optimal condition of cell growth which can be compared from the value of cell biomass produced and also the specific growth rate. Therefore, the biomass concentration of other measurement for CO_2 aeration which are 5%, 10% and 15% showed lower growth rate compared to 2% CO_2. This is because the cell cultures became acidic due too much of CO_2 provided and limit the cell assimilation. In addition, the concentration of CO_2 aeration above than 5% could be harmful and inhibit the microalgae growth. Moreover, the effects of 15% CO_2 exhibit no, or very poor growth and the other grew with extended of lag phase or decreased growth rate (Negoro *et al*., 1991). It also has been indicated that the strain of *Nannochloropsis* sp. has better growth in an enriched CO_2 aeration compared to air aeration (Chiu *et al.,* 2009). This is because there is no limitation of carbon source in growth of microalgae.

Table 4.1: Dry Weight Analysis for Carbon Dioxide Effect

Sample CO_2 aeration (%)	Time (Day)	Biomass Dry weight (g)	Biomass Concentration (g/L)	Specific Growth Rate (μD^{-1})
2	0	0.0020	0.2000	0.0000
	1	0.0026	0.2600	0.2624
	2	0.0039	0.3900	0.2027
	3	0.0048	0.4800	0.0692
	4	0.0055	0.5500	0.1361
	5	0.0062	0.6200	0.0240
	6	0.0057	0.5700	-0.0140
	7	0.0037	0.3700	-0.0864
5	0	0.0020	0.2000	0.0000
	1	0.0025	0.2500	0.2231
	2	0.0031	0.3100	0.1076
	3	0.0035	0.3500	0.0405
	4	0.0041	0.4100	0.0396
	5	0.0043	0.4300	0.0095
	6	0.0042	0.4200	-0.0039
	7	0.0031	0.3100	-0.0434
10	0	0.002	0.2000	0.0000
	1	0.0023	0.2300	0.1398
	2	0.0026	0.2600	0.0613
	3	0.0035	0.3500	0.0991
	4	0.0048	0.4800	0.0790
	5	0.0053	0.5300	0.0198
	6	0.0048	0.4800	-0.0165
	7	0.0036	0.3600	-0.0411

Sample CO_2 aeration (%)	Time (Day)	Biomass Dry weight (g)	Biomass Concentration (g/L)	Specific Growth Rate (μD^{-1})
15	0	0.0020	0.2000	0.0000
	1	0.0025	0.2500	0.2231
	2	0.0032	0.3200	0.1234
	3	0.0034	0.3400	0.0202
	4	0.0041	0.4100	0.0468
	5	0.0045	0.4500	0.0186
	6	0.0038	0.3800	-0.0282
	7	0.0033	0.3300	-0.0202

4.1.2 Effect of Light Intensity on Growth and Biomass Concentration of *Nannochloropsis* sp.

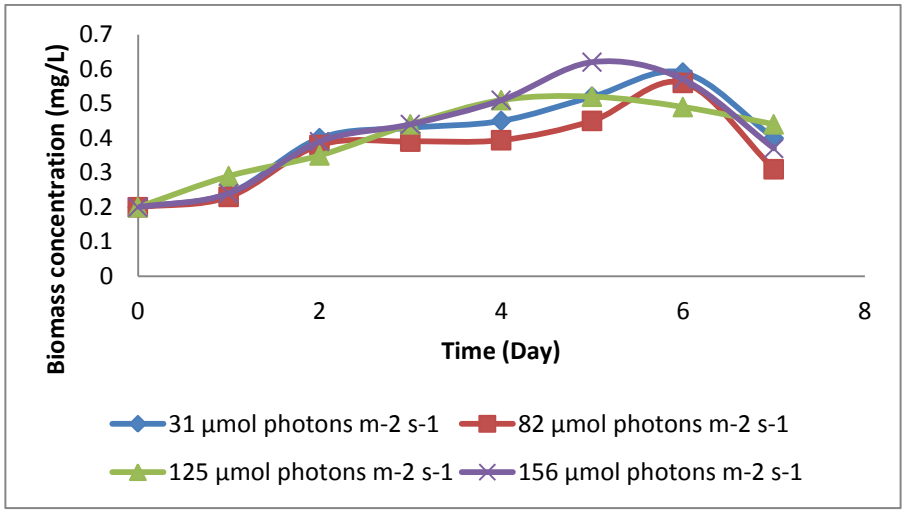

Figure 4.2: Effect of the light intensity effect on the growth of *Nannochloropsis* sp.

As the result shown in Figure 4.2, the growth of *Nannochloropsis* sp. is optimum and has the highest of biomass concentration on the highest value of the light intensity

which is 156 µmol photons m^{-2} s^{-1} than those observed at 31 µmol photons m^{-2} s^{-1}, 82 µmol photons m^{-2} s^{-1}, 125 µmol photons m^{-2} s^{-1}. The biomass concentration and cell growth is compared on the fifth day where the early of stationary phase for each sample. The biomass concentration and growth on the effect of 156 µmol photons m^{-2} s^{-1} is the highest value which are 0.6200 g/L and 0.0391µD^{-1}, respectively. Meanwhile, the specific growth of 31 µmol photons m^{-2} s^{-1}, 82 µmol photons m^{-2} s^{-1}, 125 µmol photons m^{-2} s^{-1} are 0.0289µD^{-1}, 0.0286 µD^{-1}, 0.0039µD^{-1}, respectively. Consequently, under the highest light of intensity, the percentages of neutral lipids were significantly higher (Guiheneuf *et al.,* 2009). According to Zou *et al* (2000), for a given light intensity, the light regime to which the average cell is exposed is a function of both the photosynthetic photon flux density and the cell density. Besides that, the growth profile of *Nannochloropsis* sp. shows a proportional increase with increment in light intensity. However after a certain light intensity, the growth profile shows declination with further increase in light intensity. This value of light intensity from where the declination starts is termed to cause the phenomena of light inhibition, such that the growth of *Nannochloropsis* sp. decreases due to the damage in light pigments at high intensity.

Table 4.2: Dry Weight Analysis for Light Intensity Effect

Sample (µmol photons m^{-2} s^{-1})	**Time (Day)**	**Biomass Dry weight (g)**	**Biomass Concentration (g/L)**	**Specific Growth Rate (µD^{-1})**
31	0	0.0020	0.2000	0.0000
	1	0.0024	0.2400	0.1823
	2	0.0040	0.4000	0.2554
	3	0.0043	0.4300	0.0241
	4	0.0045	0.4500	0.0114
	5	0.0052	0.5200	0.0289
	6	0.0059	0.5900	0.0210
	7	0.0040	0.4000	-0.3887

Sample (µmol photons m^{-2} s^{-1})	Time (Day)	Biomass Dry weight (g)	Biomass Concentration (g/L)	Specific Growth Rate (μD^{-1})
82	0	0.0020	0.2000	0.0000
	1	0.0023	0.2300	0.1398
	2	0.0038	0.3800	0.2510
	3	0.0039	0.3900	0.0087
	4	0.00394	0.3940	0.0026
	5	0.0045	0.4500	0.0286
	6	0.0056	0.5600	0.0364
	7	0.0031	0.3100	-0.0845
125	0	0.0020	0.2000	0.0000
	1	0.0029	0.2900	0.3716
	2	0.0035	0.3500	0.0940
	3	0.0044	0.4400	0.0763
	4	0.0051	0.5100	0.0370
	5	0.0052	0.5200	0.0039
	6	0.0049	0.4900	-0.0099
	7	0.0044	0.4400	-0.0154
156	0	0.0020	0.2000	0.0000
	1	0.0024	0.2400	0.1823
	2	0.0039	0.3900	0.2428
	3	0.0044	0.4400	0.0402
	4	0.0051	0.5100	0.0370
	5	0.0062	0.6200	0.0391
	6	0.0057	0.5700	-0.0140
	7	0.0037	0.3700	-0.0617

4.2 Analysis of Omega- 3 Fatty Acid Composition

4.2.1 Effect of Carbon Dioxide on Eicosapentaenoic Acid Concentration

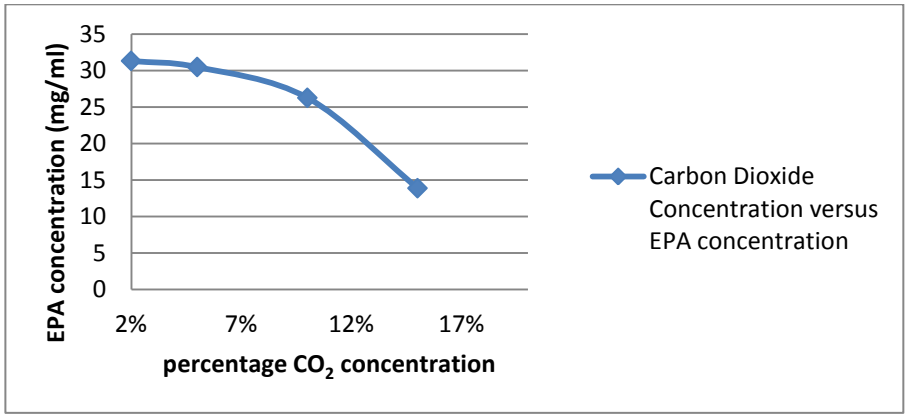

Figure 4.3: Effect of various CO_2 concentrations on EPA concentration

Table 4.3 shows the eicosapentaenoic acid (EPA) productivity as a function of the light intensity, for *Nannochloropsis* sp. cultures aerated with various CO_2 concentrations. In the semicontinuous of culture system, the *Nannochloropsis* sp. cells were collected at the end of cultivation time for determination of EPA productivity. From the graph above (Figure 4.3), it can be stated that, as increasing CO_2 concentration of aeration from 2% to 15%, the EPA productivity was generally decreasing. It seems that CO_2 transiently affects the EPA content and cells inhibited to higher CO_2 concentration. The cultured aerated with 2% CO_2 showed an optimal growth potential and has higher value of EPA concentration which is 31.3121 mg/ml. The EPA content proportionally increases as the biomass concentration increase. This is due to the cell assimilation level in converting the nutrients into biomass in the algae cell. Therefore, the optimal aeration which is 2 % CO_2 show the best result for EPA content because there is no limitation for the cell in utilizing the available nutrients into lipid biomass containing EPA. Comparing to other aeration level, which are 5%, 10% and 15%, the graph showed there are declining of EPA content as increasing the aeration percentage. This is because the algae cell of *Nannochloropsis* sp.

already inhibit at the CO_2 aeration of 5%, 10% and 15% and cannot do any biosynthesis process in the cell.

Sample	Concentration of Eicosapentaenoic acid (mg/ml)
2% Carbon Dioxide	31.3121
5% Carbon Dioxide	30.4738
10% Carbon Dioxide	26.2560
15% Carbon Dioxide	13.8789

Table 4.3: Concentration of Eicosapentaenoic acid for Carbon Dioxide Effect

4.2.2 Effect of Light Intensity on Eicosapentaenoic Acid Concentration

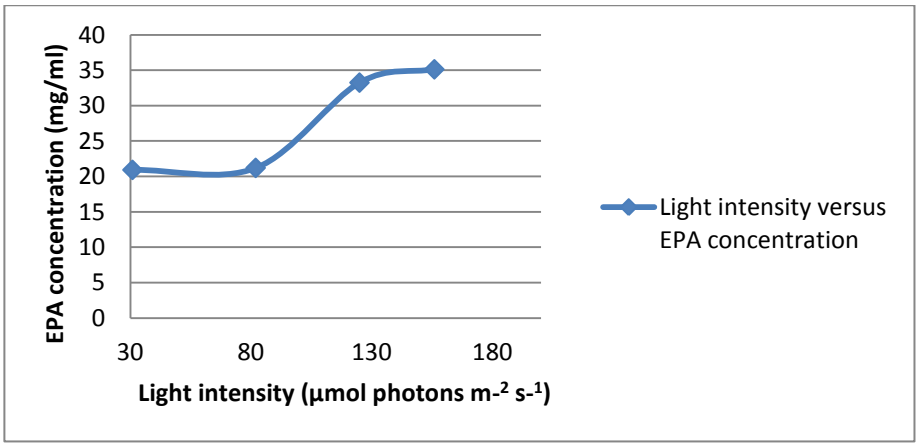

Figure 4.4: Effect of various light intensities on EPA concentration

Meanwhile, the results reported in Table 4.4 show the EPA contents on difference of light intensity that aerated with 2% of CO_2. Based on the result obtained, the productivity of EPA was more sensitive to the variations in light intensity compared to the carbon source. The EPA concentrations were significantly lower under growth-limiting irradiance than under the other light intensities. As decreasing the light intensity for

Nannochloropsis sp. cells growth, the content of EPA also declining. From the graph (Figure 4.4), the highest light intensity has the highest EPA productivity which is 35.1339 mg/ml at light intensity of 156 µmol photons m^{-2} s^{-1}. Consequently, 156 µmol photons m^{-2} s^{-1} of light intensity has the highest productivity of EPA and there is small decrease in the proportion of EPA for other measurement of light intensity (Guiheneuf *et al*., 2009). The omega-3 fatty acid containing EPA also increases in photosynthetic photon flux density resulted in a significant increase in culture density (Zou *et al*., 2000). It is worth noting that the maximal culture content of EPA in the ultrahigh cell density cultures reported was higher by applying high light intensity for photosynthesis process.

Sample	**Concentration of Eicosapentaenoic acid (mg/ml)**
31 µmol photons m^{-2} s^{-1}	20.9314
82 µmol photons m^{-2} s^{-1}	21.2135
125 µmol photons m^{-2} s^{-1}	33.2592
156 µmol photons m^{-2} s^{-1}	35.1339

Table 4.4: Concentration of Eicosapentaenoic acid for Light Intensity Effect

CHAPTER 5

CONCLUSION AND RECOMMENDATION

As a conclusion, the production for the study of omega-3 fatty acid from *Nannochloropsis* sp. on the effects of carbon dioxide and light intensity was successfully achieved. This study was done by two parameters which are carbon dioxide and light intensity in order to identify the optimum condition for producing high quantity of omega-3 fatty acid. The highest productivity of eicosapentaenoic acid by *Nannochloropsis* sp. had been obtained at 2% of carbon dioxide aeration and 156 µmol photons m^{-2} s^{-1} which are 31.3121 mg/ml and 35.1339 mg/ml, respectively. Hence, the omega-3 fatty acid accumulation of *Nannochloropsis* sp. could be increased under their optimal conditions which are in maximal efficiency of carbon dioxide and high light intensity. Besides, the recommendation for this case study is using other strain of microalgae that has the highest of lipid content among microalgae species which is *Neochlorosis oleoabundans*, thus the production of omega-3 fatty acid could be increased in the future.

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photobioreactors. *Chemical Engineering and Processing 48 (2009) 306–310* , 306–310.

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APPENDIX

6.1 Analysis of Gas Chromatography

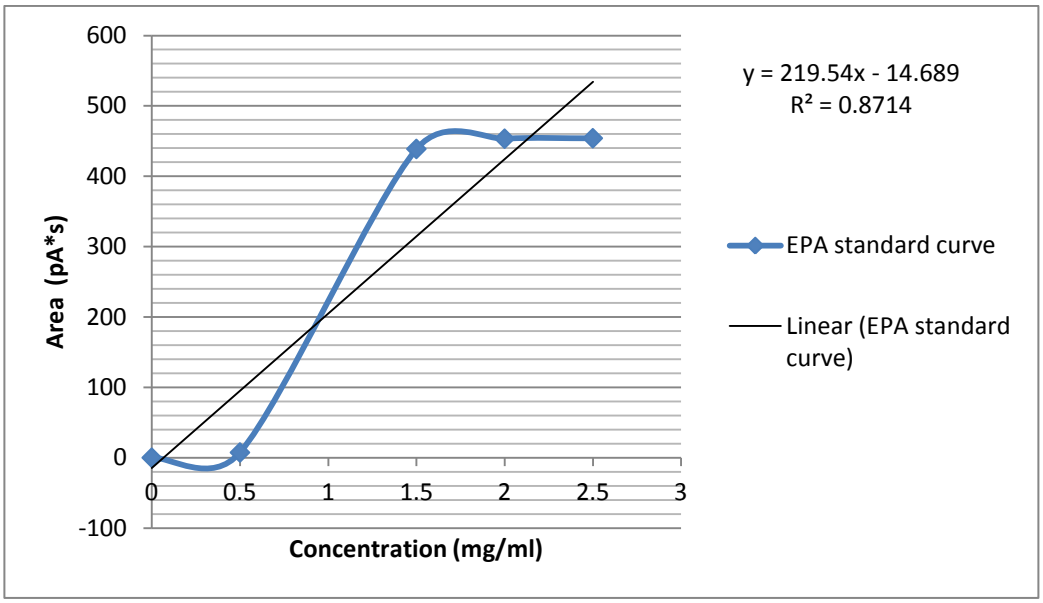

Figure 6.1: Standard Curve for Eicosapentaenoic Acid

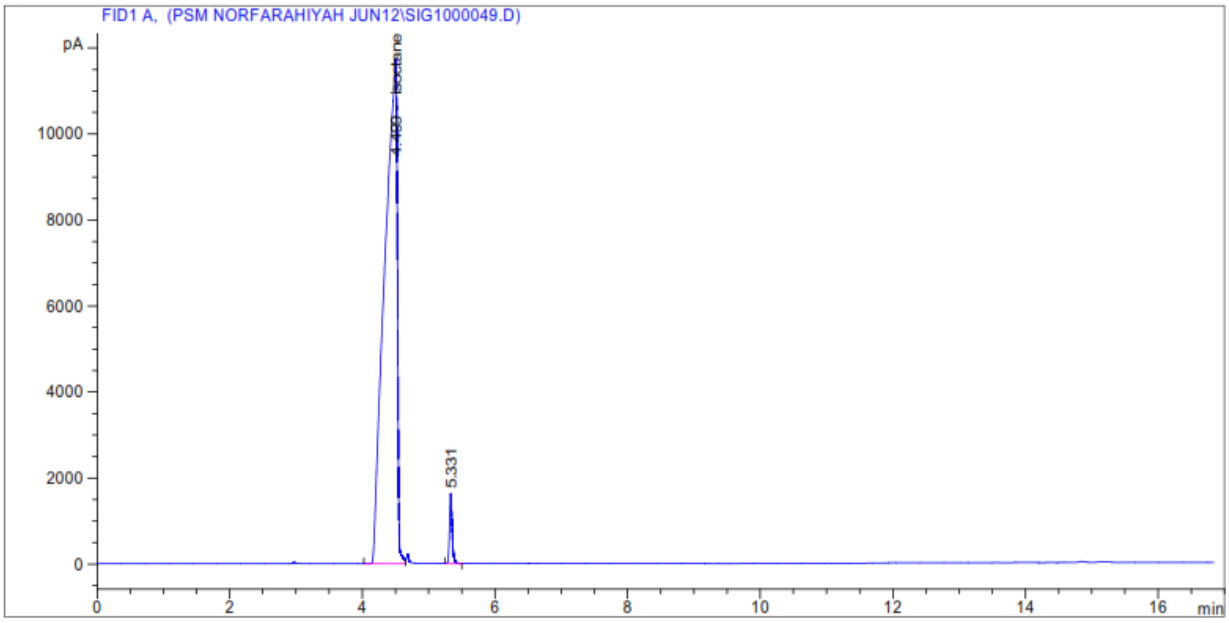

Figure 6.2: Sample of 31 µmol photons m^{-2} s^{-1}

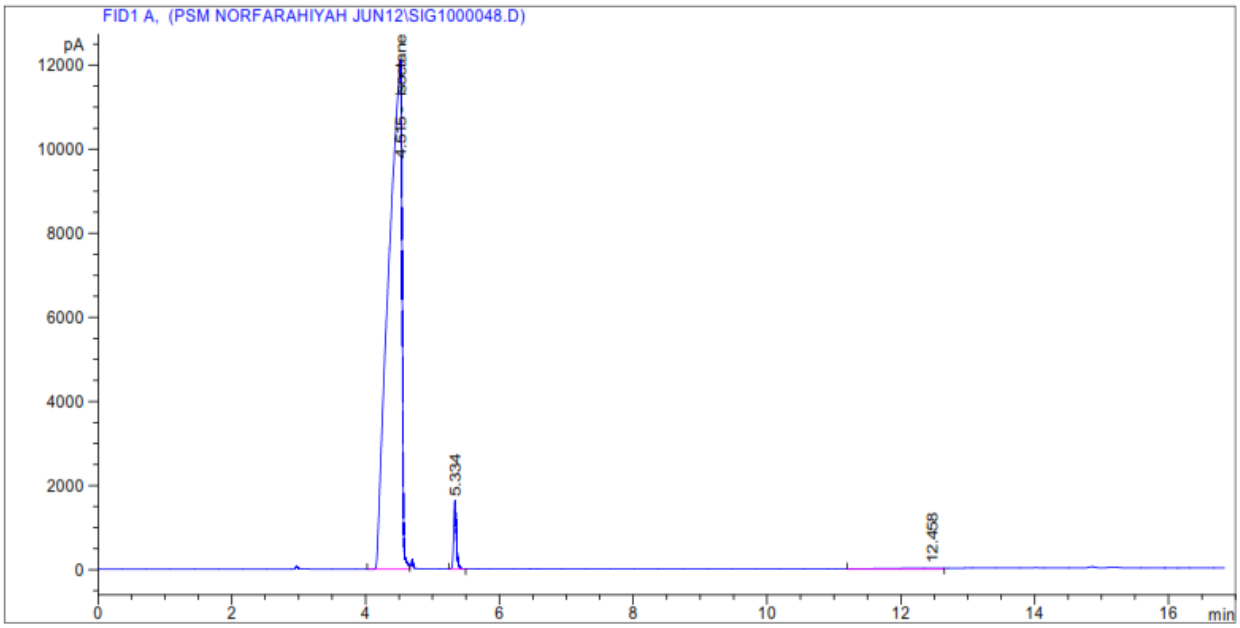

Figure 6.3: Sample of 82 µmol photons m^{-2} s^{-1}

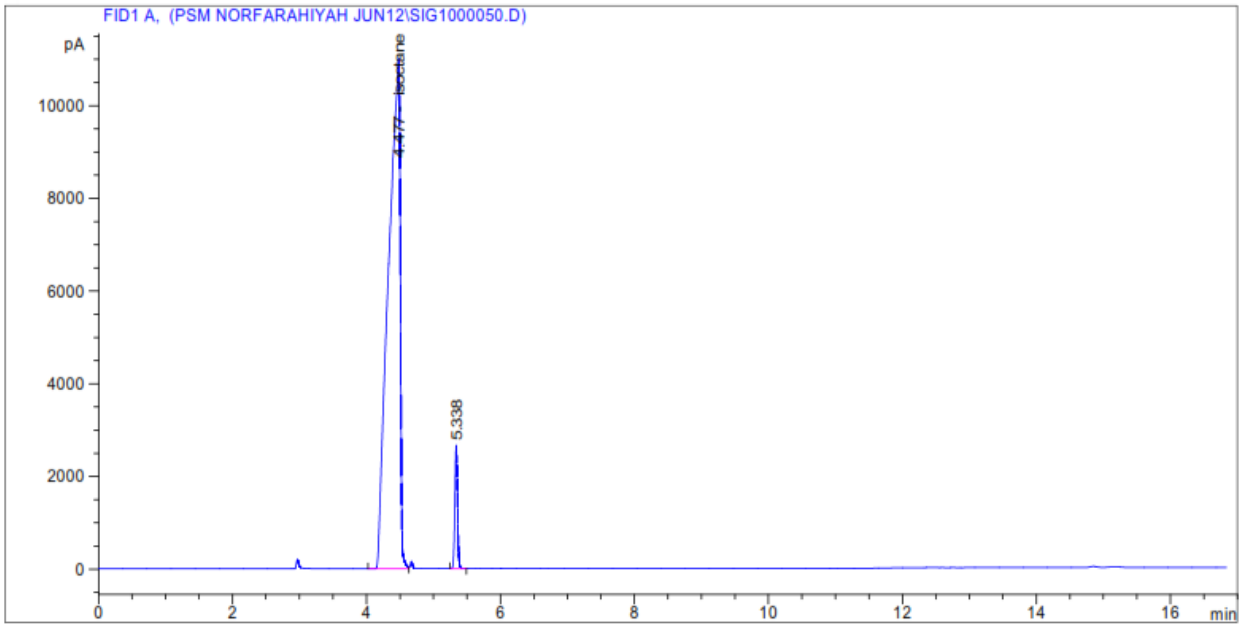

Figure 6.4: Sample of 125 µmol photons m^{-2} s^{-1}

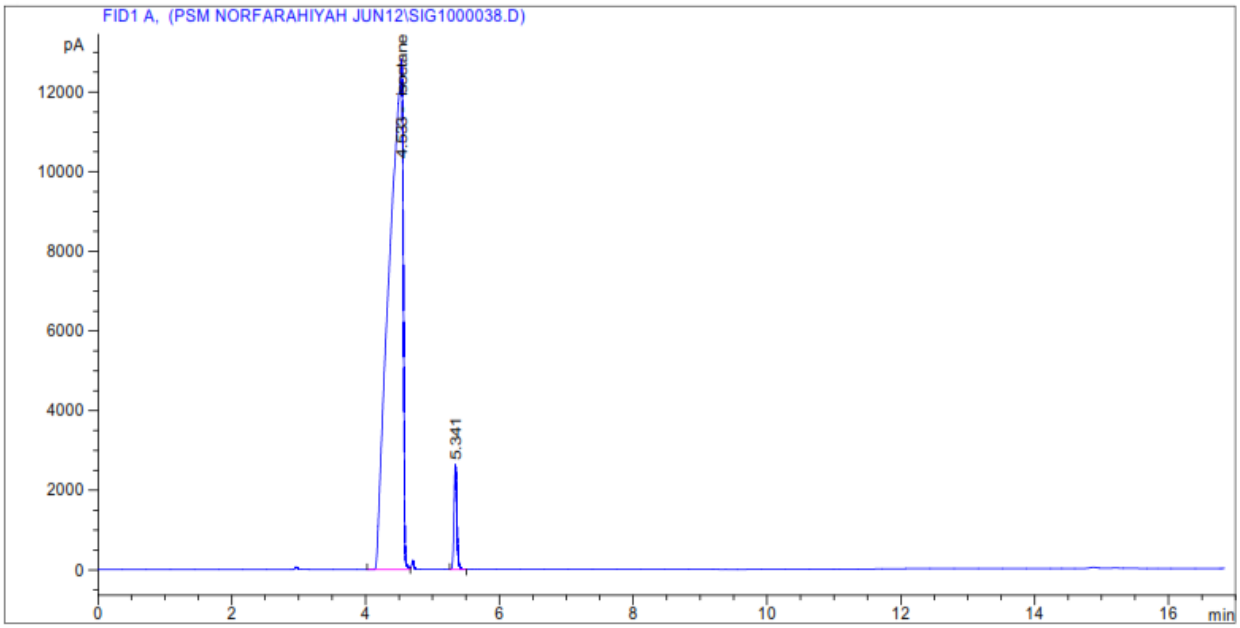

Figure 6.5: Sample of 156 µmol photons m^{-2} s^{-1}

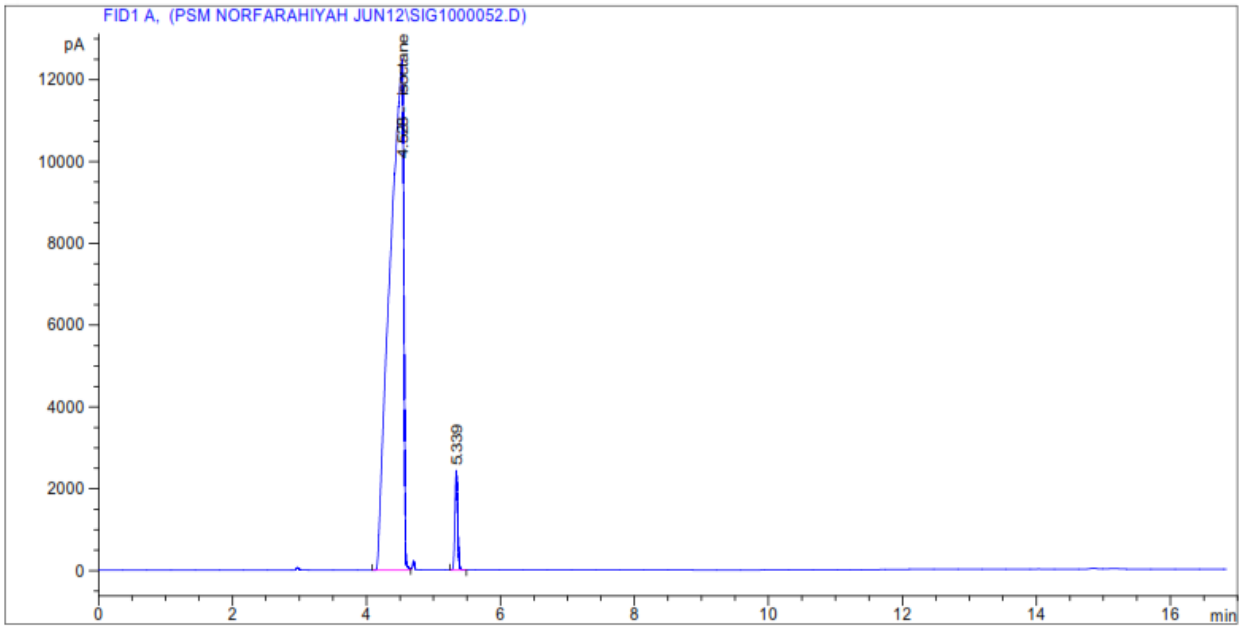

Figure 6.6: Sample of 2% Carbon Dioxide

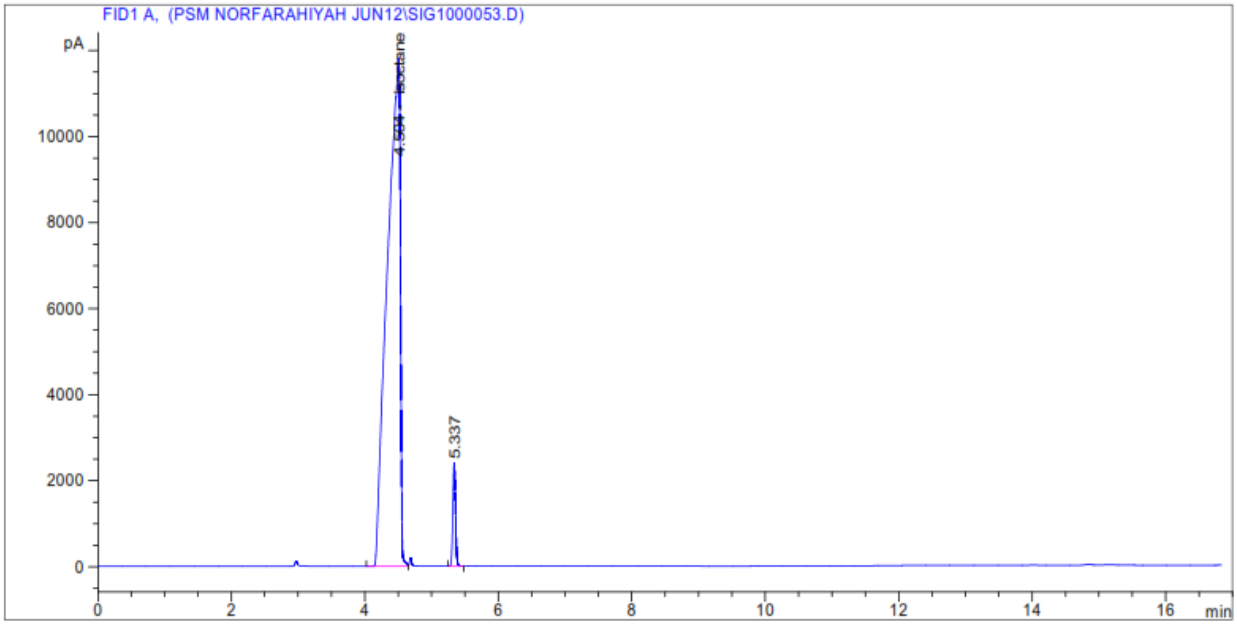

Figure 6.7: Sample of 5% Carbon Dioxide

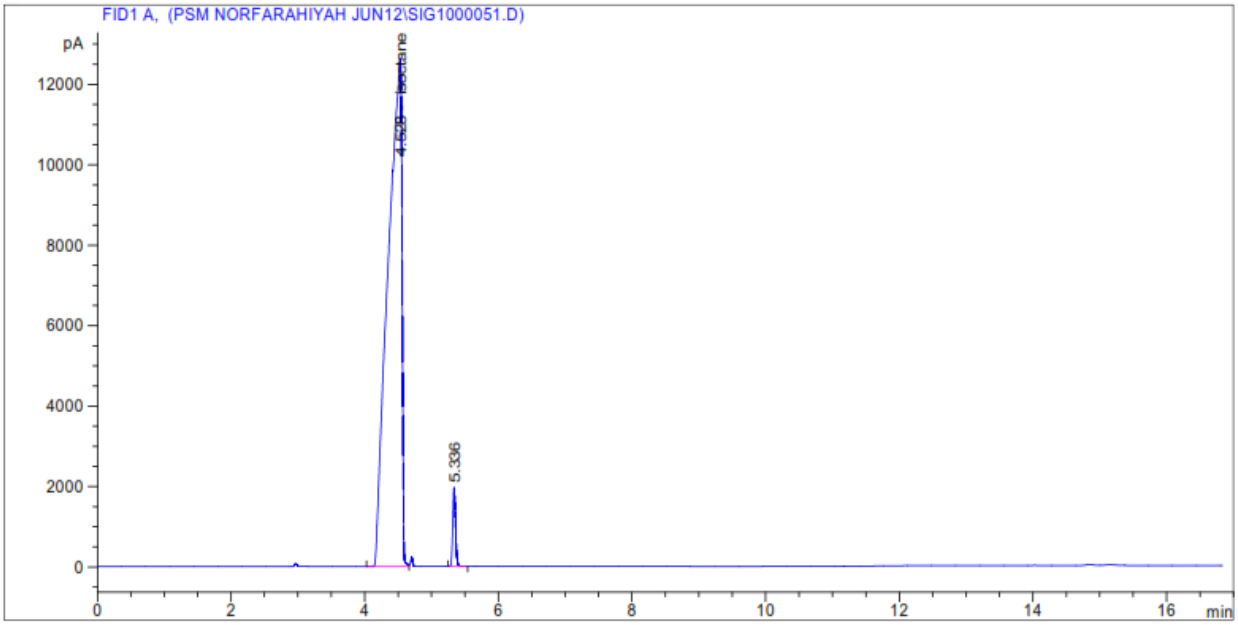

Figure 6.8: Sample of 10% Carbon Dioxide

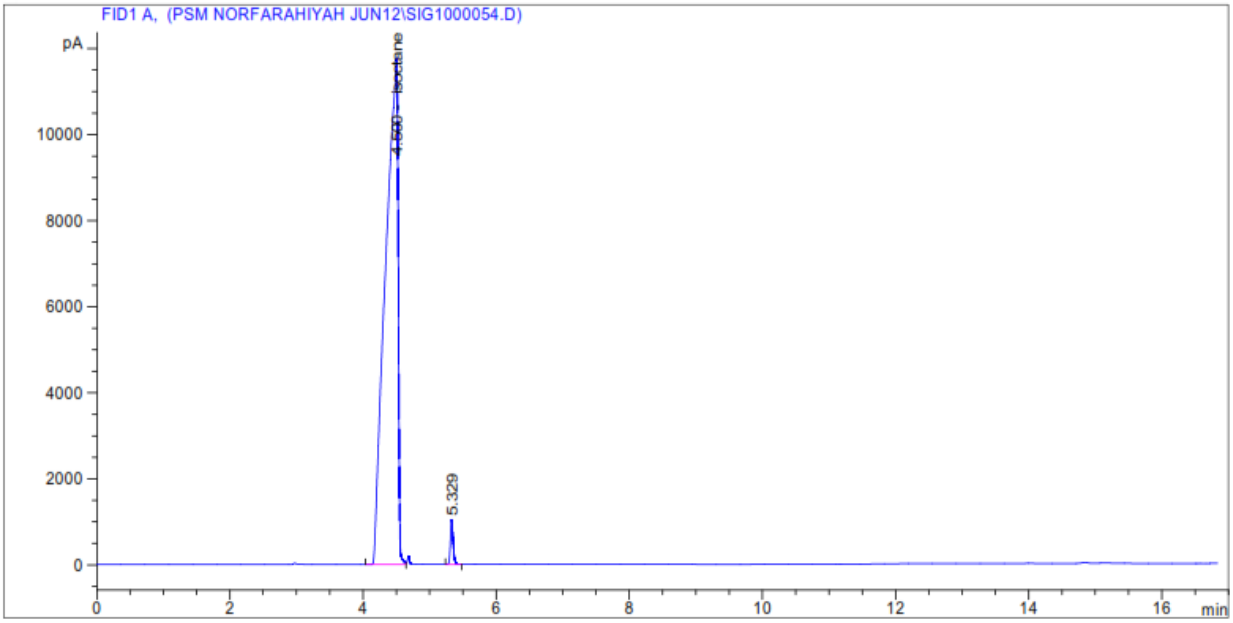

Figure 6.9: Sample of 15% Carbon Dioxide