CHAPTER 2

Literature review

2.1 CO_2 emissions and global climate conditions

Since the industrial revolution era, the lives of humankind have become easier and more convenient. Many new forms of technology have been developed to serve human demands. But these new technologies have resulted in a number of bad effects to our earth, such as in the way of coal-fired power plants and cars. These activities burn a lot of fossil fuels and discharge a huge amount of carbon dioxide (CO_2) into the atmosphere, which has lead to global warming.CO_2 is one of the main greenhouse gases that can trap heat in the atmosphere and can keep the earth warm. Besides CO_2 there are a number of the main greenhouse gases, e.g., methane (CH_4), nitrous oxide (N_2O), chlorodifluoromethane ($CHClF_2$), dichlorodifluoromethane (CCl_2F_2) and Sulfur hexafluoride (SF_6) (Table 2.1). These gases also contribute to the global warming.

Table 2.1 The main greenhouse gases and their anthropogenic source and GWP

Greenhouse gas	Chemical formula	Anthropogenic source	Global warming potential (GWP)*
Carbon dioxide	CO_2	- Fossil fuel combustion - Land use conversion - Cement production	1
Methane	CH_4	- Fossil fuels - Rice paddles - Waste dumps - Livestock	21

Table 2.1 (continued)

Greenhouse gas	Chemical formula	Anthropogenic source	Global warming potential (GWP)*
Nitrous oxide	N2O	- Fertilizer - Industrial process combustion	310
Chlorodifluoromethane, HCFC-22	$CHClF_2$	- Liquid Coolants	1,300-1,400
Dichlorodifluoromethane, CFC-12	CCl_2F_2	- Liquid Coolants - Foams	6,200-7,100
Sulfur hexafluoride	SF_6	- Dielectric fluid	23,900

*GWP for 100-year time horizon

Source: IPPC (1996)

GWP is heat-trapping capability index that measures the potential of each greenhouse gas to trap heat in the atmosphere. This capability is relative to a reference gas such as CO_2 (whose GWP is standardized at 1) over a specified time horizon. Table 2.1 shows that CH_4 has the potential to trap heat at 21 over a 100-year period and N_2O has the potential of 310, conversely SF_6 has a GWP of 23,900 over 100 years. When atmospheric greenhouse gas concentrations increase, the temperature in the atmosphere also increases. As a result, the temperature on the surface of the earth will warm up and contribute to climate change.

CO_2 is a major greenhouse gas that is diffused through human activities. Since the industrial revolution, the concentration of CO_2 in the atmosphere has already risen from 280 to 390 ppm (Figure 2.1A). Human activities that are related to CO_2 emissions between 2000 and 2010 were the highest in history, contributing to CO_2 levels in the atmosphere that are unprecedented in at least 800,000 years. Figure 2.1B shows that the anthropogenic CO_2 emissions from fossil fuels and cement production in 2010 reached 35 $GtCO_2$/yr. During 1750 and 2011, anthropogenic CO_2 emitted to the atmosphere had increased and accumulated by up to 2040 ± 310 $GtCO_2$ and continued to grow by 2.5% per year on average over the past decade (IPCC, 2014b; Friedlingstein *et al*., 2014).

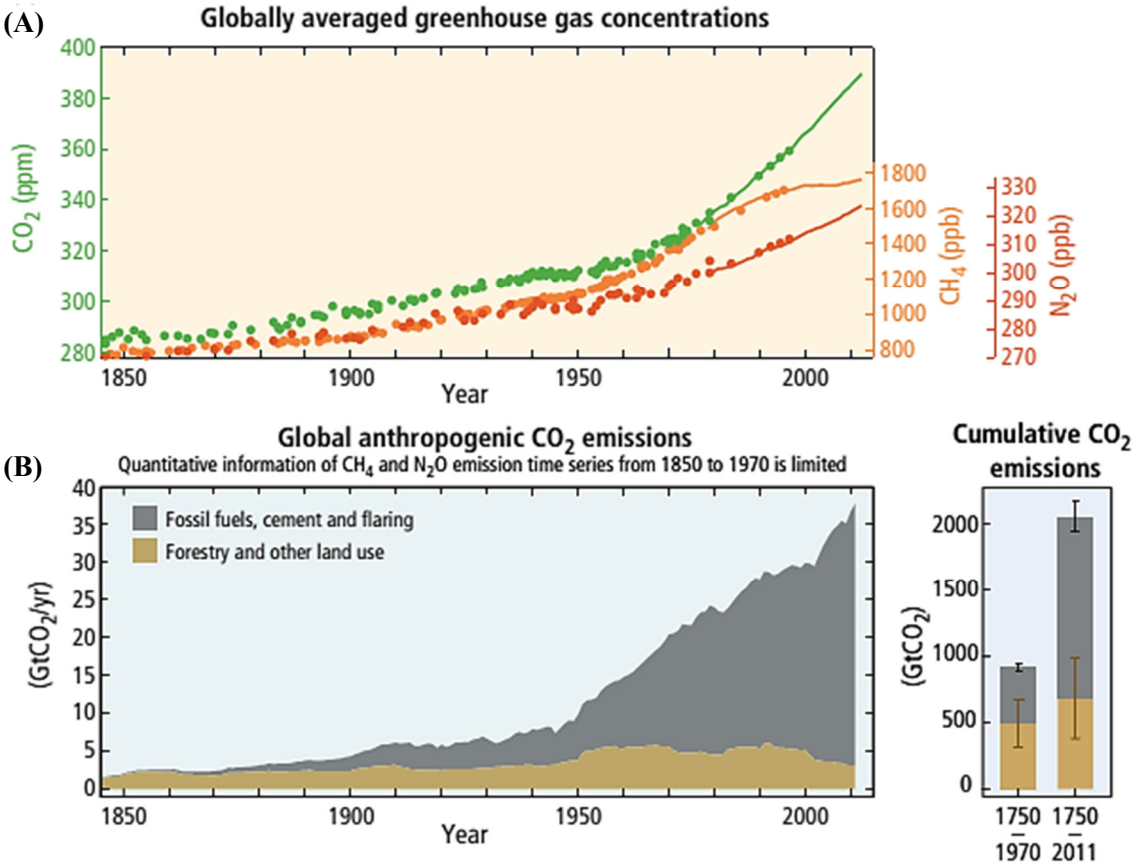

Figure 2.1 Atmospheric concentrations of greenhouse gases (A) and emissions of Global anthropogenic CO_2 levels (B)

Source: IPCC, 2014b

From 1970 to 2010, there was an increase of about 78% in the total greenhouse gas emissions. This was related to CO_2 levels accumulating through the consumption of fossil fuels and by the cement industry and flaring (IPCC, 2014a). From 1880 to 2012 the average temperature on the surface of the globe has raised to 0.85°C. This has also led to warming on the earth's surface and climate change. The impacts of climate change affects ecosystems and society in several different ways, such as it can alter worldwide temperatures, significantly influence the yields of agricultural crops, impact on human health reaction, as well as by influencing sea levels and salinity. Moreover, it may even impact on soil quality, nitrogen deposition, and plant diversity (Delcour *et al*., 2015).

2.2 CO_2 sequestration by microalgae

According to the report of IPPC, CO_2 releases from fossil fuel combustion and industrial processes increased from 420±35 $GtCO_2$ in 1750 to 1300±11 $GtCO_2$ in 2010 (IPPC, 2014a). Sequestration of CO_2 from relevant industries is very important to CO_2 mitigation in the atmosphere and it also reduces the impacts of CO_2 accumulation on climate change. There are several methods of CO_2 sequestration as are shown in Figure 2.2. The major classification system is based on non-biological and biological sequestration strategies (Nogia *et al*., 2013).

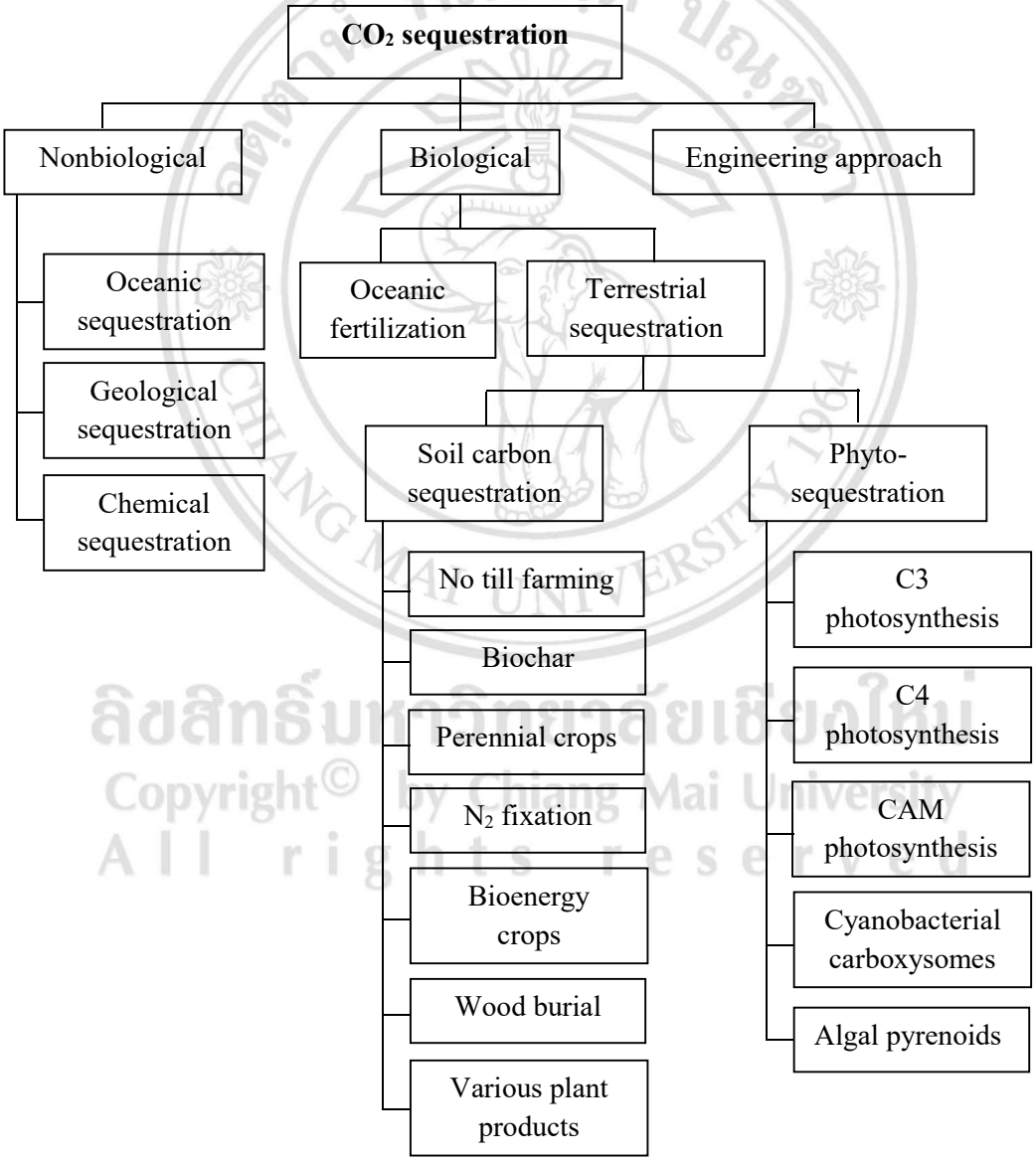

Figure 2.2 Various methods of CO_2 sequestration

Source: Nogia *et al*. (2013)

Among the various methods for CO_2 capture and storage, the biological CO_2 sequestration system using phototropic microalgae has received significant attention. When compared to other energy crops, microalgae reveal greater advantages in terms of photosynthetic efficiency, biomass production, and CO_2 fixation ability (Tang *et al*., 2011). The utilization of microalgae for CO_2 sequestration has several important advantages as follows (Kumar *et al.*, 2011; Jajesniak *et al.*, 2014).

1) Mitigating CO_2 (the primary source of global warming)
2) Wide distribution (particularly in moist environment)
3) Multiple modes of cultivation available (*e.g*., open ponds, photobioreactors).
4) Fast growing and high cell density
5) Fast CO_2 uptake
6) High cellular lipid content
7) Production of high-value byproducts (*e.g*., proteins, aquaculture nutrients, fertilizers)
8) Genetic modification tools available

Moreover, biological CO_2 fixation using microalgae is thought to be a feasible form of technology, which is both energy-saving and environmentally friendly (Tang *et al*., 2011). Several microalgae species have been studied for CO_2 reduction, such as *Chlorella kessleri*, C. *vulgaris*, *Scenedesmus obliquus*, *Spirulina* sp. (de Morais and Costa, 2007a), *Chlorella* sp. (Chiu *et al*., 2008) and *Phaeodactylum tricornutum* (Sobczuk *et al*., 2000). Pabakaran and Ravindran (2012) investigated the growth and CO_2 fixation ability of three algal strains; *Ulothrix* sp., *Chlorella* sp. and *Chlorococcum* sp. The growth rate of *Chlorella* sp. was the highest. The carbon content and CO_2 fixation rate of *Chlorella* sp. were 0.486 g dw^{-1} and 0.68 g mL^{-1} d^{-1}. This strain also displayed the highest lipid content with 24.3 ± 0.81% of dry weight. Goswami *et al*. (2012) studied the growth rate and CO_2 fixation capability of *Selenastrum* sp., after being inoculated in BG11 medium with 4.4, 5.2, 7.5 and 8.2 mg L^{-1} CO_2 concentrations. The maximum biomass productivity was 0.89 mg $mL^{-1}d^{-1}$ under 5.2 mg L^{-1} CO_2 concentrations and the lipid production was 15% of the dry weight. Furthermore, its CO_2 fixation rate was 1.67 mg $L^{-1}d^{-1}$.

2.3 Microalgae and biodiesel production

2.3.1 Lipids from microalgae

Microalgae are an oleaginous microorganism and microalgal lipids can be used as feedstock in biodiesel production (Hossain *et al*., 2008).Microalgae-based biodiesel possesses many benefits such as having a high lipid content, fast growth rate, and for being easily modified by biotechnological techniques (Table 2.2).

Table 2.2 Comparison of various oleaginous microorganisms for oil production

Microorganism	Advantages	Disadvantages
Microalgal oils	1) Fatty acid composition is similar to common plant oils 2) High oil yield (~85% w/w) under optimal conditions 3) Short-time growth cycle 4) Relatively single composition exists in microalgae	1) Properties of most algal oils are not as numerous as 'fossil diesel' 2) Microalgal cultivation cost is higher than that of common plant oils
Bacterial oils	1) Short-time growth cycle	1) Most of bacteria cannot yield lipids but complicated lipoid
Oleaginous yeasts and mildews	1) High oil yield in some species 2) Short-time growth cycle 3) High capability of growth under different cultivation conditions 4) Conversion and utilization of scrap fiber can be applied to produce useful oils and can be applied to the waste oil dealing process	1) Suitable filtration and the cultivation is required to achieve high oil yields 2) Oils extraction process is very complicated and should new technologies are needed to improve the process 3) Cultivation cost is also higher than common plant oils

Source: Huang *et al*. (2010)

Many researchers have proved that several species of microalgae can produce and accumulate high lipid content in their cells. Table 2.3 shows a comparison of lipid content and biomass productivities of various well-known microalgae.

Table 2.3 Lipid content and biomass productivities of various species of microalgae

Microalgae species	Lipid content (% of dry weight)	Biomass productivity ($g\ L^{-1}\ d^{-1}$)
Ankistrodesmus sp.	24.0-31.0	-
Botryococcus braunii	25.0-75.0	0.02
Chaetoceros muelleri	33.6	0.07
Chaetoceros calcitrans	14.6-16.4/39.8	0.04
Chlorella emersonii	25.0-63.0	0.036-0.041
Chlorella protothecoides	14.6-57.8	2.00-7.70
Chlorella sorokiniana	19.0-22.0	0.23-1.47
Chlorella vulgaris	5.0-58.0	0.03-0.20
Chlorella sp.	10.0-48.0	0.02-2.5
Chlorella pyrenoidosa	2.90-3.64	2.0
Chlorococcum sp.	0.28	19.3
Crypthecodinium cohnii	10	20.0-51.1
Dunaliella salina	6.0-25.0	0.22-0.34
Dunaliella primolecta	23.1	0.09
Dunaliella tertiolecta	16.7-71.0	0.12
Dunaliella sp.	17.5-67.0	-
Ellipsoidion sp.	27.4	0.17
Euglena gracilis	14.0-20.0	7.70
Haematococcus pluvialis	25.0	0.05-0.06
Isochrysis galbana	7.0-40.0	0.32-1.60
Isochrysis sp.	7.1-33.0	0.08-0.17
Monodus subterraneus	16.0	0.19
Monallanthus salina	20.0-22.0	0.08

Table 2.3 (continued)

Microalgae species	Lipid content (% of dry weight)	Biomass productivity ($g\ L^{-1}\ d^{-1}$)
Nannochloris sp.	20.0-56.0	0.17-0.51
Nannochloropsis oculata	22.7-29.7	0.37-0.48
Nannochloropsis sp.	12.0-53.0	0.17-0.43
Neochloris oleoabundans	29.0-65.0	-
Nitzschia sp.	16.0-47.0	-
Oocystis pusilla	10.5	-
Pavlova salina	30.9	0.16
Pavlova lutheri	35.5	0.14
Phaeodactylum tricornutum	18.0-57.0	0.003-1.9
Porphyridium cruentum	9.0-18.8/60.7	0.36-1.5
Scenedesmus obliquus	11.0-55.0	0.0004-0.74
Scenedesmus quadricauda	1.9-18.4	0.19
Scenedesmus sp.	19.6-21.1	0.03-0.26
Skeletonema sp.	13.3-31.8	0.09
Skeletonema costatum	13.5-51.3	0.08
Spirulina platensis	4.0-16.6	0.06-4.3
Spirulina maxima	4.0-9.0	0.21-0.25
Thalassiosira pseudonana	20.6	0.08
Tetraselmis suecica	8.5-23.0	0.12-0.32
Tetraselmis sp.	12.6-14.7	0.30

Source: Mata *et al*. (2010)

Microalgae cells are made up of many components. The main components are lipids. 2-70% of the lipid content by total dry weight can be extracted from the algal biomass but it does vary according to the growth conditions and species type (Table 2.3). The potential of algae-based biodiesel production depends on the selection of suitable strains in regard to profitable yields and lipid quality (Nascimento *et al*., 2012). Although the maximum biomass productivity of algae will be found under optimal cultivation conditions, it

does not serve as a guarantee of the maximum lipid content. Some species have high lipid content but their growth rate is very slow. From Table 2.3, the lipid content of *Botryococcus braunii* could reach 75% of the dry weight but it is associated with low biomass productivity. Most common microalgae (*Chlorella*, *Dunaliella*, *Isochrysis*, *Nannochloropsis*, *Scenedesmus*, *Spirulina*) possess lipid contents in a range of 15-50% but they have higher biomass productivity. This indicates that the selection of suitable strains of microalgae is very important. Some factors such as the ability of algae under specific environmental conditions and capability to uptake nutrient by algae should be considered before selecting the most suitable strains or sufficient species for use as biodiesel feedstock (Mata *et al*., 2010).

2.3.2 Biosynthesis of microalgal lipids

Generally, photosynthetic pathway in the green algae are similar to C_3 higher plants. In recent year, some researchers suggested that some species of green algae could perform C_4 photosynthesis (C_4 plant e.g. maize and sugarcane) (Xu *et al*., 2012). C_3 photosynthesis is a "normal" photosynthesis, it only uses the Calvin cycle for fixing CO_2, uses RuBisCO (ribulose 1, 5-bisphosphate carboxylase) as a catalytic enzyme and the primary CO_2 acceptor is Ribulose bi phosphate (RUBP). For C_4 photosynthesis, Pyruvate orthophosphate dikinase (PPDK) and RuBisCO were used to generate PEP, the primary CO_2 acceptor is phosphoenol pyruvic acid (PEP) (Wang *et al*., 2012; Xu *et al*., 2012).

In the photosynthetic process of green microalgae (C_3 photosynthesis), they fix CO_2 in the atmosphere and produce sugar by using sunlight as an energy source and water as an electron source. All reactions can be summarized as follows:

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

There are two steps of photosynthesis, a light-dependent reaction (light reaction) and a light-independent reaction (dark reaction). Light reaction is the step that captures the energy of light to produce the cofactors, ATP

(adenosine-5′-triphosphate) and NADPH (nicotinamide adenine dinucleotide phosphate). These products are used to capture and reduce CO_2 for the dark reaction step. In the dark reaction step, sugars are produced using the carboxylase activity of the enzyme RuBisCO and ATP. This step is also called the Calvin cycle (Longnergan, 2000; Kumar *et al.*, 2011). After the Calvin cycle, the chloroplasts generate the 3-phosphoglycerate (3PGA) with CO_2 followed by the glycolytic pathway to form pyruvate (Figure 2.3). Pyruvate releases CO_2 and constructs acetyl-CoA (acetyl coenzyme) as the precursor for fatty acid (the building blocks for triacylglycerols, TAGs, and all other cellular lipids) synthesis in the chloroplast (Fan *et al*., 2011).

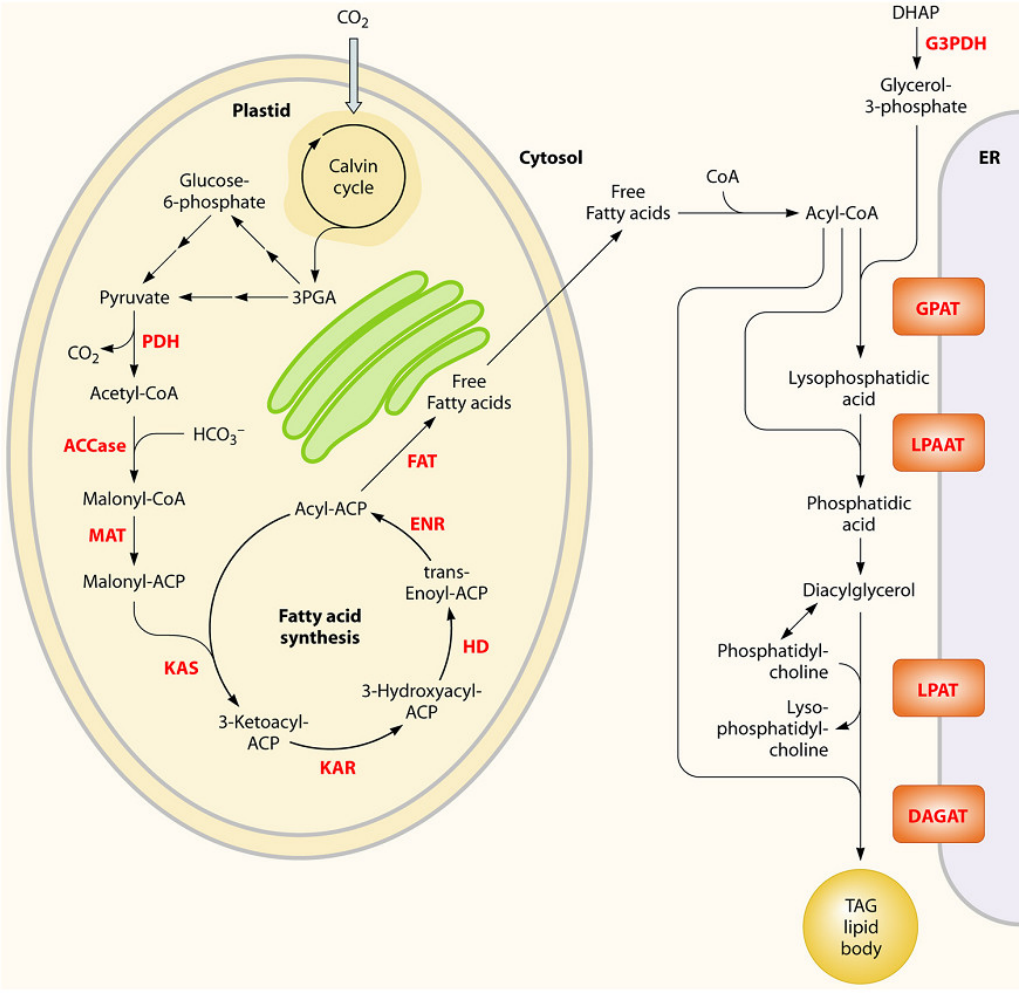

Figure 2.3 Simplified overview of the metabolites and representative pathways in microalgal lipid biosynthesis

Source: Radakovits *et al*. (2010)

Triglycerides or TAGs are the main substrate for use in the biodiesel production process. TAG is an ester that consists of a glycerol (a carbohydrate) backbone attached with three long chains of fatty acids. The three fatty acids can be comprised of many types because the chain lengths of fatty acids are varied and contain 16-20 carbon atoms. The common saturated and unsaturated fatty acids found in plants and animals could be found in some species of microalgae. The most common saturated fatty acids are palmitic acid (C16:0) and stearic acid (C18:0). The most common unsaturated fatty acids are oleicacid (C18:1), linolenic acid (C18:2) and linolenic acid (C18:3) (Khan *et al*., 2009; Yoo *et al*., 2010).

2.3.3 Biodiesel from microalgae

Biodiesel refers to plant oil- or animal fat- or algal lipid-based diesel fuel. It consists of fatty acid mono-alkyl esters. Biodiesel is produced though a chemical reaction involving the mixture of TAGs, alcohol and the catalytic process known as ‘‘transesterification’’. This reaction consists of multiple steps to convert TAGs into biodiesel and glycerol (by product) (Van Gerpen, 2005; Khan *et al*., 2009). Figure 2.4 shows the overall process of transesterification, where R_1, R_2 and R_3 are long-chain fatty acids containing hydrogen and carbon atoms.

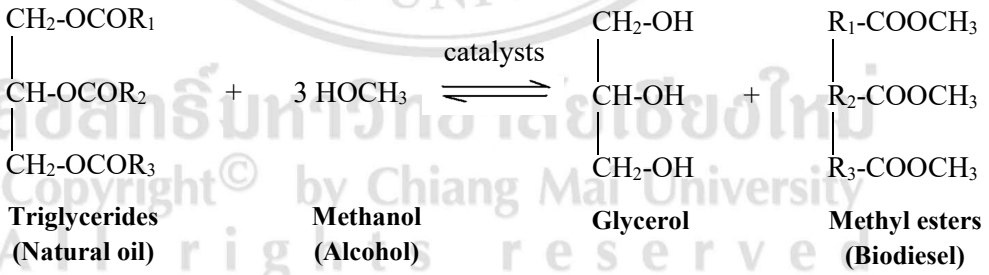

Figure 2.4 Overall reaction of transesterification

Source: Khan *et al*., 2009

Microalgae are the anticipating source for biodiesel production and have potential to replace ‘fossil diesel’. When compared with other types of oil crops, microalgae possess higher oil yield and they do not require a large land area for cultivation. Microalgae also have more advantages in terms of

photosynthetic efficiency, CO_2 fixation ability, and growth rate than oil plants (Chisti, 2007; Kirrolia *et al*., 2013). Table 2.4 shows a list of the various sources for biodiesel production including microalgae. Utilization of plant-based biodiesel to replace 50% of the 'fossil diesel' used in the U.S. transport sector requires large land areas that are presently used for food production (Kirrolia *et al*., 2013). Microalgae show higher potential in producing biodiesel than most other plants. 7.6-15.2 million hectares of cultivating area can be used to produce biodiesel to account for 50% of the 'fossil diesel' production. They also produce more than 35,000 L of microalgal oil per hectare of land, which is 100-fold higher than that of soybeans, the dominant U.S. biodiesel feedstock.

Table 2.4 Oil yields of various sources for biodiesel production

Source	Oil yield ($L\ ha^{-1}$)	Required land area (M ha)*
Corn	172	1,540
Soybean	446	594
Canola	1,190	223
Jatropha	1,892	140
Coconut	2,689	99
Oil palm	5,950	45
Microalgae**	70,405	7.6
Microalgae***	35,202	15.2

* To meet 50% of all transport fuel needs of U.S.

** 40% oil (% of dry weight) in biomass

*** 20% oil (% of dry weight) in biomass

Source: Kirrolia *et al*. (2013)

When compared with the ASTM biodiesel standard, biodiesel from microalgae have similar properties with the standard biodiesel, except for its flash point (Table 2.5).

Table 2.5 Properties of biodiesel from microalgae compared with conventional diesel fuel and ASTM biodiesel standard

Properties	Biodiesel from microalgae	Diesel fuel	ASTM biodiesel standard
Density (kg L^{-1})	0.864	0.838	0.84-0.90
Viscosity (mm^2 s^{-1}, cSt at 40°C)	5.2	1.9-4.1	3.5-5.0
Flash point (°C)	115	75	min 100
Solidifying point (°C)	-12	-50 to 10	-
Cold filter plugging point (°C)	-11	-3 (max -6.7)	summer max 0 winter max < -15
Acid value (mg KOH g^{-1})	0.374	max 0.5	max 0.5
Heating value (MJ k g^{-1})	41	40-45	-
H/C ratio	1.81	1.81	-

Source: Huang *et al*. (2010)

However not all strains of microalgae can be used to produce biodiesel. It depends on their lipid productivity (Ngoc *et al*., 2013). The screening and isolation of fast-growing and lipid-rich microalgae has been receiving attention. Many species of microalgae suitable for producing bio-oil have been identified. For example, *Chlorella sorokiniana* isolated from a turbid, open pond in KwaZulu-Natal, South Africa, showed the highest biomass and lipid content (0.218 g L^{-1} and 61.52% of dry weight) under conditions involving wastewater supplemented with urea (Ramana *et al*., 2015). Mandal and Mallick (2012) reported that *Scenedesmus obliquus* can grow well when cultured in the recirculatory aquaculture system using the mixture of two wastes, fish pond discharge and poultry litter, as the nutrient source. After 11 cultivation cycles per year, *S. obliquus* could produce 14,400 liters of lipids per hectare per year.

Jena *et al.* (2012) studied the suitability of three isolated strains of microalgae; *Chlorococcum* sp., *Chlorella* sp. and *Scenedesmus* sp, for the production of biodiesel. They found that *Scenedesmus* sp. seems to be the most suitable strain for biodiesel production. The biomass concentration and lipid

productivity of this strain were 0.9 g L^{-1} and 24.66 mg L^{-1} d^{-1}, respectively. In the fatty acid composition results, *Scenedesmus* sp. also was found to be the best material for biodiesel production with a high value of palmitic acid and some unsaturated fatty acids (e.g. C18:1 and C18:2). Girisha *et al*. (2014) also reported that *Scenedesmus* sp. is suitable for biodiesel production. They found that this alga had higher levels of biomass (4.533 $mgmL^{-1}$), lipids (49%), myristic acid (9.0%), oleic acid (9.3%), linolenic acid (20.1%), palmitic acid (35.3%), and stearic acid (6.1%) than *Botryococus* sp. and *Chlorella* sp. Zhou *et al*. (2011) found that five strains of microalgae; *Chlorella* sp., *Heynigia* sp., *Hindakia* sp., *Micractinium* sp., and *Scenedesmus* sp., display the adaptive ability to grow under concentrated municipal wastewater (CMW). These strains also have high growth rates (0.455-0.498d^{-1}) and high lipid productivity levels (74.5-77.8 mg L^{-1} d^{-1}).

2.4 Microalgae cultivation for CO_2 capture and biodiesel production

Microalgae have high potential for both CO_2 capture and biodiesel production (Yoo *et al*., 2010; Chiu *et al*., 2011; Kumar *et al*., 2011). CO_2 sources and CO_2 levels play an important role in CO_2 capture, as well as the biomass and lipid accumulation of microalgae. However, the successful mitigation of CO_2 and biodiesel production using microalgae requires suitable strains that can grow under high CO_2 level conditions, and can grow faster and can accumulate high lipid content (Tang *et al*., 2011; Almarales *et al*., 2012).

2.4.1 CO_2 sources

Generally, microalgae cultivation uses carbon dioxide from three sources. The first source is atmospheric CO_2 (0.03% v/v). Another source is CO_2 that comes from various industrial gases such as flaring gas and exhaust gas. The last source is CO_2 that is chemically fixed in the form of soluble carbonates such as Na_2CO_3 and $NaHCO_3$ (Znad *et al*., 2012). Atmospheric CO_2 is probably the most basic source for microalgae cultivation because algae can consume CO_2 for normal growth during photosynthesis. However, the atmospheric concentrations of CO_2 (0.03% v/v) are insufficient for the

purposes of promoting the high growth rate of microalgae and the high rate of lipid productivity that is needed for large-scale biofuel production. In contrast, the industrial exhaust gases that are a result of combustion processes display better recovery figures due to higher CO_2 concentrations (15-20% v/v) (Ono and Cuello, 2003; Kumar *et al*., 2010).

Direct utilization of industrial exhaust gases in algae-based fuel production has been considered. One of the advantages of using exhaust gases for microalgal cultivation is the reduced cost of CO_2 separation from the exhaust gases (Beneman *et al*., 1987). Many reports have suggested exhaust gases as a carbon source for microalgae, which could combine biofuel production with current CO_2 mitigation strategies. For example, Brown (1996) found that *Monoraphidium minutum* (NREL strain Monor02) could grow well in exhaust gas (simulated) containing 13.6% CO_2, with the balance of N_2 when compared with ambient air. The green microalga *Chlorella* sp. strain MTF-7 was cultivated using direct exhaust gas aeration (23% v/v CO_2). This gas was constructed from the oven of a steel plant. After 6 days of cultivation, the dry weight, biomass productivity and lipid content of the alga were 2.87 g L^{-1}, 0.52 g $L^{-1}d^{-1}$ and 25.2% of the dry weight, respectively (Chiu *et al*., 2011). Yoo *et al*. (2010) investigated the algal growth and lipid production using exhaust gases. Two types of microalgae; *Botryococcus braunii* and *Scenedesmus* sp., were cultivated with real exhaust gas containing 5.5% CO_2. The biomass productivity levels for *B. braunii* and *Scenedesmus* sp. with real exhaust gas were 77 and 203 mg L^{-1} d^{-1}, respectively and the lipid content was recorded as 24% and 18% of the dry weight, respectively.

2.4.2 Selection of microalgal strains

Microalgae are photosynthetic microorganism containing a variety of strains. There are about 200,000-800,000 species of microalgae that exist in the Earth's ecosystems, and around 35,000 species have been studied and analyzed (Ebenezer *et al*., 2011). Not all of these species are suitable for mitigation of CO_2 and biodiesel production. Therefore, the selection of suitable microalgae strains that can tolerate high CO_2 levels, grow fast and

produce high lipid contents are of great importance (Tang *et al*., 2011).To capture the CO_2 efficiently, the microalgal strains should possess the following characteristics; a high CO_2 fixation rate, a high tolerance of trace components of exhaust gases such as SO_x and NO_x, a high tolerance to harsh conditions (temperature, pH, light), a high potential for providing useful by-products such as biofuel and the ability to be collected during the harvest process (Sen, 2012). Moreover, the selection of suitable CO_2 tolerance strains has a significant impact on cost competitiveness and the efficacy of the biological CO_2 mitigation process (Brennan and Owende, 2010).

Generally, the CO_2 tolerance of microalgae is dependent upon cell density, pH, nutrients, light and species (Van Den Hende *et al*., 2012). Many researchers have reported that several species of microalgae can tolerate and grow well in high CO_2 concentrations of up to 10-100% v/v (Table 2.6).

Table 2.6 CO_2 tolerance of various microalgal species

Algal species	Maximum CO_2 tolerance	Reference
Cyanidium caldarium	100 %	Seckbach *et al*. (1971)
Scenedesmus sp.	80 %	Hanagata *et al.* (1992)
Chlorococcum littorale	60 %	Kodama *et al.* (1993)
Synechococcus elongatus	60 %	Miyairi (1997)
Euglena gracilis	45 %	Nakano *et al.* (1996)
Chlorella sp.	40 %	Hanagata *et al.* (1992)
Eudorina spp.	20 %	Hanagata *et al.* (1992)
Spirulina sp.	18 %	de Morais and Costa (2007a)
Dunaliella ertiolecta	15 %	Nagase *et al.* (1998)
Nannochloris sp.	15 %	Yoshihara *et al.* (1996)
Chlamydomonas sp.	15 %	Miura *et al.* (1993)
Tetraselmis sp.	14 %	Matsumoto *et al.* (1995)
Botryococcus braunii	10 %	Boonma *et al.* (2014)

As shown in Table 2.6, many microalgae were tested under high concentrations of over 10% v/v CO_2. For example, *Cyanidium caldarium*

could grow in 100 % CO_2 (Seckbach *et al*., 1971). One of the high CO_2 tolerant species is *Chlorococcum littorale*. This species could be grown under 60% CO_2 using the stepwise adaptation technique (Kodama *et al*., 1993). The two strains of green microalgae *Scenedesmus* sp. and *Chlorella* sp. could grow well under 80% and 40% CO_2 concentrations, respectively. But the highest growth rate was found under 10-20 % CO_2 (Hanagata *et al.*, 1992). *Euglena gracilis* is one of the high CO_2 tolerant species, it was observed under 5-45 % concentrations of CO_2. At 5% CO_2 concentration, the best growth was obtained (Nakano *et al*., 1996). *Botryococcus braunii*, a rich-hydrocarbon microalga, could grow under 10% CO_2 supplement and revealed higher lipid content (41.74% of dry weight) than that of the 0.03% and 1% CO_2 supplemented specimens (Boonma *et al.,* 2014). Moreover, de Morais and Costa (2007a) found the cyanobacterium, *Spirulina* sp. could grow under 18% CO_2 concentration but the maximum biomass concentration (4.13 g L^{-1}) was found with 6% CO_2 supplement.

2.5 Factors affecting the cultivation of microalgae

There are several factors affecting algae growth and its ability to photosynthesize, as well as its potential for conversion into algae biomass and lipids. Significantly, microalgal biofuel production systems can be organized and controlled. The parameters such as temperature, light, nutrient content, pH and CO_2 concentrations can be observed and optimized for high biomass and lipid productivity (Zeng *et al*., 2011).

2.5.1 CO_2 concentrations

CO_2 is the primary source of carbon for microalgal growth. Microalgal biomass consists of approximately 50% carbon of the dry cell weight. All of this carbon is usually received from CO_2 (Chisti, 2007). When CO_2 is dissolved in an aqueous environment, it always remains in equilibrium with H_2CO_3, CO_3^{2-} and HCO_3^- and its concentration is dependent on the temperature and pH of the solutions (Kumar *et al*., 2011). Microalgae can accumulate HCO_3^- by at least 20-fold over ambient CO_2 levels (Moroney and Somanchi, 1999). Atmospheric CO_2 (0.03% v/v) is not adequate to promote

lipid productivity and high growth rate of microalgae. Increases of CO_2 concentrations can enhance the efficiency of microalgal growth and metabolism (Kumar *et al*., 2010).

There have been many studies on the growth characteristics, lipid production and CO_2 fixation of microalgae under high CO_2 concentrations. Ge *et al*. (2010) investigated the growth of *Botryococcus bruanii* 765 under 2-20% CO_2 aeration. They found that this alga could grow well under all tested CO_2 concentration levels. The maximum biomass and lipid content were 2.31 g L^{-1} and 12.71 % of the dry weight at 20% CO_2aeration.The effect on growth of *Scenedesmus* sp. and *Chlorella* sp. under four CO_2 concentrations (6, 12, 18 and 24% of CO_2) was studied. Under aeration with 24% CO_2, the maximum biomass concentrations were observed in both strains with 0.12 g L^{-1} of *Scenedesmus* sp. and 0.2 g L^{-1} of *Chlorella* sp. (Makarevičienė *et al*., 2011). Tang *et al.* (2011) found that *Chlorella pyrenoidosa* SJTU-2 and *Scenedesmus obliquus* SJTU-3 could grow well under CO_2 concentrations in a range of 5-20%v/v CO_2(>1.22 g L^{-1}). These algae could grow under 50% CO_2 (>0.69 g L^{-1}). High CO_2 levels (30–50%) were suitable for the enhancement of microalgal lipid accumulation. Yue and Chen (2005) investigated the effects of CO_2 levels on the growth of *Chlorella* sp. strain ZY-1. This strain was isolated from fresh water at the Shenyang thermal power plant in China. The alga was cultivated under high CO_2 concentrations ranging from 0% to 70%. Under aeration with 10% and 20% CO_2, a high growth rate was observed. At 10% CO_2, the growth rate was recorded as 1.17 g L^{-1} d^{-1}. After 6 days of cultivation, the cell concentration achieved 5.772 g L^{-1}. Furthermore, Yun *et al*. (1997) studied the growth and CO_2 fixation ability of *Chlorella vulgaris* under 15% CO_2. After being adapted to 5% (v/v) CO_2, the growth of *C. vulgaris* in 15% (v/v) CO_2 was greater than that of the specimens adapted to air with the highest growth rate and CO_2 fixation levels of 1.49 g dm^{-3} and 26 g CO_2 m^{-3} h^{-1}, respectively.

2.5.2 Temperature

The main factor that is important for microalgal growth is temperature. It has significant impacts on the chemical compositions of the algal cells, morphological, physiological activity, the absorption of nutrients and CO_2 fixation of various microalgal species. Low temperatures resulted in the inhibition of microalgal growth. On the other hand, higher temperatures normally increase the metabolic rates of microalgae (Kumar *et al*., 2010). The effects of temperature on microalgal growth were found to be species-dependent. The optimum temperature for *Chlorella vulgaris* ARC 1 was found to be in the range of 25 to 30°C. At 30°C, the maximum biomass production and chlorophyll content of the microalga was found at 6% v/v CO_2 supplement (Chinnasamy *et al*., 2009). Many researches found that the optimal cultivation temperature for the specific growth rate of *Nannochloropsis oculata* and *Nitzschia laevis*, were reported as 20°Cand 23°C, respectively (Converti *et al*., 2009; Chen *et al*., 2009 and Westerhoff *et al*., 2010). Fu *et al*. (2008) studied the effect of different CO_2 and temperature levels on the growth rate of two algal species, the raphidophyte *Heterosigma akashiwo* and the dinoflagellate *Protocentrum minimum.* They found that at 750 ppm CO_2 and 24°C, the biomass productivity and the chlorophyll content of the two species were higher than that of 375 ppm CO_2 and 20°C.

The temperature of the exhaust gas released from power plants and other similar sources was approximately 120°C. The potential for CO_2 capture from these gases depends on selection of the thermophilic species and the heat control system applied (Kumar *et al*., 2011). Many species have been identified as species that can tolerate high temperatures up to 50°C. Ono and Cuello (2007) studied the growth of the *Chlorogleopsis* sp. (SC2), a thermophilic cyanobacterium originally isolated from the Yellowstone National Park. Cyanobacterium grew very well at high temperatures up to 50°C with 5% CO_2 and the biomass produced by this strain was recorded at 1.4 g L^{-1}. *Chroccoccus* sp., *Geitlerinema sulphureum* and *Planktolyngbya crassa* showed temperature tolerance up to 42°C, though the optimum growth

was observed at a temperature of 32°C (Manjre and Deodhar, 2013). Furthermore, Beklee *et al*. (2014) reported that 21 thermotolerant strain isolates out of 130 samples collected in Spain, Germany, Portugal and Italy could be grown at 40°C or above. The 13 identified strains were genetically recognized to be part of the Family Chlorellaceae. Béchet *et al*. (2013) found that the temperature-tolerant *Chlorella sorokiniana* could grow in an outdoor column photobioreactor without temperature control (the operating temperature was raised up to 41°C).

2.5.3 pH

pH is an important parameter for regulating microalgae growth. Most microalgal species can grow well in neutral pH values, whereas the optimum growth rate of some species can be found under lower and higher pH values. For example, *Chlorococcum littorale* (Sasaki *et al*., 1998) and *Spirulina (Arthrospira) platensis* (Kim *et al*., 2007) display optimum growth rates under pH values of around 5.5 and 9.5, respectively. The value of pH in the microalgal cultivation system is influenced by the concentration of CO_2 or CO_3^{2-}. Carbonic acid (H_2CO_3) is formed after CO_2 dissolved in the water and the pH values are decreased. However, both biomass and lipid productivity can be raised as the CO_2 concentration level is increased, but this can also reduce the pH, which has a strong effect on microalgae growth as it can affect the activity of different enzymes (Ying *et al*., 2014). In contrast, microalgae can use HCO_3^- as a carbon source, which can reduce the pH value by changing CO_2 (Brewer and Goldman, 1976). Many researchers have studied the effects of CO_2 levels and pH values on microalgal growth and biofuel production. Olivieri *et al*. (2012) investigated the effects of both CO_2 concentration and the pH of the medium on biodiesel production by *Stichococcus bacillaris* ACUF 158/11. They found that the cultivation of algae with 5% CO_2 (pH 7) in bubble columns showed higher biomass and lipid productivity levels (256 and 80 mg $L^{-1}d^{-1}$) than in the vertical bubble column (124 and 42 mg L^{-1} d^{-1}). *Nannochloropsis salina* showed the highest growth rates at pH values of 8 and 9 under 400 ppm CO_2 supplement. In terms of the results of lipid

accumulation, there was no difference in the pH 8 treatment and the un-buffered controls (the pH fluctuated between pH 7.81 and 9.70). The lipid content of *N. salina* in the pH 8 treatment was 24.75 %, compared to 21.8 % in the un-buffered controls (Bartley *et al*., 2014). Spilling *et al*. (2012) found lipid accumulation of the diatom *Phaeodactylum tricornutum* CCAP was reduced when the pH value was increased to higher levels. Sukkrom *et al*. (2015) observed that *Ankistrodesmus* sp. seemed to grow best in the medium with a pH of 8. Moreover, pH ranging from 6 to 8 was not found to have significant influences on biomass and the lipid productivity of microalgae.

2.5.4 Light

Light intensity is an environmental factor that plays an important role in the growth and CO_2 fixation of microalgae. In photosynthesis production, microalgae require a light/dark system. They require a light system to produce organic cofactors (ATP and NADPH) and require a dark system to synthesize biomolecules for algae growth (e.g. carbohydrates, protein and lipid) (Al-Qasmi *et al*., 2012). The light intensity levels for the inhibition and saturation of algal growth depend upon the propriety of other environmental factors such as CO_2 concentration, temperature, and nutrient supplementation (Sorokin and Krauss, 1958). When compared with higher plants, the light intensity requirements of microalgae are very low. However the photosynthetic productivity of microalgae raised when the intensity of light was risen up to 400 μmol m^{-2} s^{-1} (Munõz and Guieysse, 2006). Observations of photoinhibition were done during the peak hours of sunny days when the light intensity can increase to 4000 μmol m^{-2} s^{-1} (Fuentes *et al*., 1999). Cheirsilp and Torpee (2012) found that when the intensity of light was increased from 2000 to 8000 lux, the level of growth of *Chlorella* sp. also increased. On the other hand, the growth of *Nannochloropsis* sp. continuously increased when the intensity of light was increased up to 10,000 lux. Ho *et al*. (2012) studied the impact of light intensity on microalgal growth and lipid/carbohydrate production. They observed that the biomass productivity and lipid/carbohydrate production of the *Scenedesmus obliquus* CNW-N was

highest under a light intensity of 400 μmolm^{-2} s^{-1}. Furthermore, *S. obliquus* was also studied at different light intensities in a flat plate photobioreactor in a range of 10-1000 μmolm^{-2} s^{-1}. This microalga showed the optimum growth rate at 150 μmolm^{-2} s^{-1} while the protein, carbohydrate, and lipid contents of the algae cells showed no major variations at the different light intensities. The lipid contents recorded under all conditions were similar at about 40% of the dry weight (Gris *et al*., 2014).

2.5.5 Nutrient requirements

In addition to CO_2 and light considerations, microalgae require nutrients for efficient algal growth. Nitrogen (N) and phosphorus (P) are the main elements that play a significant role in the growth of microalgae. Also, trace elements (e.g. magnesium and iron) are essential elements for algal growth. These elements are important to generate a balanced medium for optimum growth and CO_2 fixation of the microalgae (Zeng *et al*., 2011; Prabakaran and Ravindran, 2012).

Nitrogen is a major essential nutrient for microalgal growth. Microalgae can use several sources of nitrogen, for example, nitrite, nitrate, and ammonium ion. Moreover, they also utilize organic nitrogen compounds such as peptides, free amino acids, and urea to synthesize amino acids and proteins (Vonrückert and Giani, 2004). Nitrogen has certain significant effects on algal growth and lipid productivity. Surendhiran and Vijay (2014) reported that the growth of *Nannochloropsis oculata* was increased under nitrogen rich conditions. In contrast, the nitrogen-starved cells contained more lipid droplets with increased cell size than was found in the nitrogen rich cells. The lipid content was calculated as 33.18% and 54.26% for the nitrogen rich cells and the nitrogen starved cells. Chu *et al*. (2014) reported that nitrogen starvation compared to nitrogen repletion enhanced the biodiesel productivity of *S. obliquus*. Nitrogen depletion could induce lipid accumulation in *Chlamydomonas* sp. strain JSC4.The algae lipid content was raised rapidly from 15.3 to 41.1% (Ho *et al*., 2014). Additionally, Widjaja *et al*. (2009)

found that a lack of nitrogen over a long period of time noticeably leads to greater accumulation of lipids inside the cells of *Chlorella vulgaris*.

Phosphorus is another essential nutrient for microalgal growth. Phosphate (PO_4) is necessary for the formation of phospholipids, nucleic acids and ester phosphates such as phosphorylated sugars in algal cells. Furthermore, PO_4 plays an important role in the metabolic pathway of algae in the form of ATP and NADPH (Roopnarain *et al*., 2014). The limitation of phosphorus is an environmental force that can raise lipid production in microalgal cells. Feng *et al*. (2012) found that the lipid content of *Chlorella zofingiensis* cultured in P-deficient medium (44.7%) was higher than that of the P-sufficient medium (33.5%). *C. protothecoides* displayed the maximum lipid productivity level of 224.14 mg L^{-1} d^{-1} when it was cultivated with a combination of N-deficiency and P- deficiency conditions (Li *et al*., 2014). Xin *et al*. (2010) found that under conditions of nitrogen or phosphorus limitations, the *Scenedesmus* sp. LX1 could accumulate high levels of lipids at 30% and 53% of the dry weight, respectively.

Trace elements (e.g. Fe, Mn, Mg and Zn) are also essential nutrients that can affect microalgal growth. The supplementation of an important trace element can affect algal growth by limiting the growth rate, the total yield or both (Spencer, 1957). A positive influence on cell growth of the mixed microalgae (cyanobacteria and Chlorophytes) appeared along with a raise of the trace element concentration levels (Zn, Fe and Fe in range from 0.001~0.03, 30.01~0.20 and 0.01~0.13, mg L^{-1}, respectively). On the other hand, there was a negative influence found with a rise in the concentration levels of Zn, Fe and Mn to above 0.03, 0.20 and 0.13, mg L^{-1}, respectively) (Zhihong *et al*., 2010). Song *et al*. (2012) reported the maximum biomass concentration of the *Botryococcus bruanii* strain UTEX 572 was observed at an optimal level of micronutrients at 0.266 mM Fe, 0.707 mM Mn, 0.624 mM Mo and 3.38 mM Ni. In contrast, the maximum hydrocarbon content was found in the medium that consisted of 10.43 mM Fe, 6.53 mM Mn, 0.012 mM Mo and 1.73 mM Ni. Furthermore, Lui *et al*. (2008) investigated the impact of iron

on the growth and lipid production of *Chlorella vulgaris*. The alga was cultivated in the culture medium that contained Fe^{3+}levels in the range of 0, 1.2 x 10^{-8}, 1.2x 10^{-7}, 1.2 x 10^{-6} and 1.2 x 10^{-5} mol L^{-1}. The results showed that the lipid content of *C. vulgaris* cultured with 1.2 x 10^{-5} mol L^{-1} $FeCl_3$ was increased up to 56.6% of the dry weight and was 3 to 7-fold higher than in the experiments with the lower Fe^{3+} levels.