CULTIVATION OF *CHLORELLA VULGARIS* USING ORGANIC FERTILIZER AS NUTRIENT SOURCE FOR BIODIESEL， MALTODEXTRIN PRODUCTION AND CO_2-BIOMITIGATION

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by

LAM MAN KEE

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This thesis is dedicated to my beloved parents and brothers for their persistent support, keen advice and numerous help.

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LIST OF ABBREVIATIONS

AFEX	Ammonia fiber explosion
Aq	Aqueous
BBM	Bold’s Basal Medium
BOD	Biochemical oxygen demand
CCM	Carbon concentrating mechanism
CO	Carbon monoxide
CO_2	Carbon dioxide
COD	Chemical oxygen demand
EER	Energy efficiency ratio
ELSD	Evaporative light scattering detector
EPS	Extracellular polymer substances
EU	European Union
FAEE	Fatty acid ethyl esters
FAME	Fatty acid methyl esters
FID	Flame ionization detector
FFA	Free fatty acid
FFB	Fresh fruit bunch
FT-IR	Fourier Transform Infrared Spectrometer
GC	Gas chromatography
GC-MS	Gas chromatography–mass spectrometry
GHG	Greenhouse gas
HP-LC	High performance liquid chromatography
ILAC	International Laboratory Accreditation Cooperation
LCA	Life cycle assessment
LED	Light emitting diode
LHV	Low heating value
MEA	Monoethanolamine
NREL	National Renewable Energy Laboratory
PGPB	Plant-growth promoting bacteria
RMSD	Root mean square deviation
US	United States

LIST OF SYMBOLS

A	Asymptotic of ln X_t/X_o as t decreases indefinitely
A_{IS}	Peak area of internal standard (methyl heptadecanoate)
A_o	Free CO_2 (mg/L)
B	B is the relative growth rate at time M (day^{-1})
B_o	Bicarbonate alkalinity (mg/L)
C	Asymptotic of ln X_t/X_o as t increases indefinitely
C_{carbon}	Carbon content of the microalgae cell (% w/w)
C_e	CO_2 concentration in the liquid equalized with that in gas phase (mol/L)
C_{IS}	Concentration of the internal standard solution (mg/mL)
C_L	CO_2 concentration in the liquid (mol/L)
C_o	Carbonate alkalinity (mg/L)
C_{pi}	Specific heat capacity of reactant and solvent (kJ/kg.^oC)
C_R	Capacity ratio
C_T	Total carbon concentration (mol/L)
E_c	Energy content (kJ/g) of biodiesel or maltodextrin
$E_{consumed}$	Energy consumption per 1 MJ electricity produced (MJ)
E_{e-}	Electricity conversion factor
E_{quip}	Energy input for equipment (MJ)
E_{output}	Energy output (MJ)
H	Henry coefficient (kPa/mol.L)
k	Shape parameter
K^A_{La,CO_2}	Liquid volumetric mass transfer coefficient for absorption of CO_2 (minute)
M	Time at which the maximum growth rate is reached (day)
M_c	Molecular weight of carbon
M_{CO_2}	Molecular weight of CO_2
m	Mass of the biodiesel sample (mg)
$m_{biodiesel}$	Weight of biodiesel produced (kg)
m_i	Weight of reactant and solvent involved in reaction (kg)
m_j	Amount of utility consumed
N_1	Biomass (g/L) at time t_1

N_2	Biomass (g/L) at time t_2
η	Average electricity generation efficiency (%)
OD_{540}	Optical density at wavelength 540 nm
P	Biomass productivity (g/L/day)
$P_{equipment}$	Power of equipment (Watt)
P_o	Partial pressure of CO_2 (kPa)
Q	Energy input for transesterification and hydrolysis reaction (kJ)
R	CO_2 fixation rate (g/L/day)
R^2	Coefficient of determination
S^2	Variance
T	Total alkalinity (mg/L)
t	Residence time (day)
V_{IS}	Volume of the internal standard solution used (L)
μ	Specific growth rate
μ_{max}	Maximum specific growth rate (day^{-1})
ν	Shape parameter
ΔT	Change in temperature (^oC)
Δt	Working time of equipment (seconds)
λ	Lag phase (day)

PENGKULTURAN *CHLORELLA VULGARIS* MENGGUNAKAN BAJA ORGANIK SEBAGAI SUMBER NUTRIEN UNTUK PENGHASILAN BIODIESEL, MALTODEKSTRIN DAN BIO-PENGURANGAN CO_2

ABSTRAK

Dalam kajian ini, baja organik yang berasal dari kompos telah digunakan sebagai sumber nutrien alternatif kepada baja kimia untuk pengkulturan *Chlorella vulgaris*. Kajian ini menunjukkan bahawa *Chlorella vulgaris* bertumbuh baik dengan bekalan 100 mL medium baja organik (kandungan nitrat 26.67 mg/L), 24 jam pendedahan kepada cahaya yang berterusan dan pH 5. Biojisim mikroalga yang berjumlah 0.50 g/L boleh dicapai selepas 12 hari pengkulturan. Kadar pertumbuhan *Chlorella vulgaris* didapati meningkat dengan peningkatan kepekatan CO_2, tetapi, kecekapan penyingkiran CO_2 didapati berkurangan. Kecekapan penyingkiran CO_2 yang tertinggi, 92.2%, dapat dicapai dengan menggunakan udara persekitaran yang mengandungi 0.03% CO_2. Di samping itu, dengan menggunakan pelarut Bligh dan Dyer (nisbah metanol kepada kloroform pada 2:1), 18% lipid boleh diekstrakkan daripada biojisim kering *Chlorella vulgaris*. Sebahagian besar lipid tersebut terdiri daripada asid lemak tak tepu, seperti C18:1, C18:2 dan C18:3. Melalui kajian parameter tindak balas transesterifikasi, 95% asid lemak metil ester (FAME) atau biodiesel telah diperolehi dengan keadaan tindak balas berikut: nisbah molar metanol kepada THF kepada lipid pada 60:15:1, 21 % berat H_2SO_4, suhu 60 ℃ dan 3 jam masa tindak balas. Tambahan pula, karbohidrat yang masih terkandung dalam sisa biojisim mikroalga selepas pengekstrakan lipid telah berjaya dipulihkan untuk penghasilan maltodekstrin (produk sampingan). 90% maltodekstrin boleh dihasilkan dengan menggunakan 3 % isipadu H_2SO_4 (atau 0.56 M), pada suhu 90°C dan masa hydrolisis selama 1 jam. Selain itu, skala pilot pengkulturan *Chlorella vulgaris* dengan 100 L photobioreaktor penyekat berturutan juga telah dijalankan dalam kajian ini. Kuantiti tertinggi biojisim mikroalga yang dihasilkan apabila dikultur dalam persekitaran dalaman dan luaran adalah 0.52 g/L dan 0.28 g/L, masing-masing. Walaupun kuantiti biojisim mikroalga yang dihasilkan adalah rendah pada persekitaraan luaran, namun, nisbah kecekapan tenaganya adalah 3.3 kali lebih tinggi daripada pengkulturan dalaman. Akan tetapi, kedua-dua kaedah pengkulturan dalaman dan luaran didapati mempunyai imbangan tenaga yang negatif untuk penghasilan mikroalga biodiesel. Anggaran minimum kos pengeluaran mikroalga biodiesel dalam kajian ini adalah RM 237/L, iaitu lebih tinggi berbanding dengan harga diesel petrol semasa (RM 3.6/L). Sebaliknya, anggaran kos pengeluaran biojisim mikroalga kering adalah RM 46/kg, menunjukkan harga yang lebih rendah berbanding dengan pengkulturan menggunakan baja kimia (RM 111/kg) serta harga pasaran semasa biojisim *Chlorella* (RM 145/kg). Pertumbuhan *Chlorella vulgaris* di dalam kajian ini didapati mematuhi model Richards, dengan nilai R^2 yang tertinggi serta memaparkan nilai RMSD dan varians yang terendah.

CULTIVATION OF *CHLORELLA VULGARIS* USING ORGANIC FERTILIZER AS NUTRIENT SOURCE FOR BIODIESEL, MALTODEXTRIN PRODUCTION AND CO_2-BIOMITIGATION

ABSTRACT

In the present study, attempt was made to solve the problems by cultivating *Chlorella vulgaris* using organic fertilizer (derived from compost) instead of depending on chemical fertilizer. Under the supplement of organic nutrients, it was found that *Chlorella vulgaris* grown favourably with 100 mL of organic fertilizer medium (or corresponded to nitrate content of 26.67 mg/L), 24 hours of continuous illumination and pH of 5. About 0.50 g/L of biomass yield was attained after 12 days of cultivation. Increasing the CO_2 concentration to the cultivation could accelerate the growth of *Chlorella vulgaris,* however, reducing the CO_2 removal efficiency. The highest CO_2 removal efficiency, 92.2 %, was achieved by using atmosphere air (0.03 % of CO_2). By using Bligh and Dyer extraction solvents (methanol to chloroform volume ratio of 2:1), about 18 % of lipid can be extracted from the dried *Chlorella vulgaris* biomass. The lipid was mainly comprised of unsaturated fatty acids, such as C18:1, C18:2 and C18:3. Through transesterification reaction parametric study, about 95 % of fatty acid methyl ester (FAME) or biodiesel was attained under the following conditions: methanol to THF to lipid molar ratio of 60:15:1, H_2SO_4 concentration of 21 wt.%, temperature of 60 ℃ and reaction time of 3 hours. In addition, the carbohydrate left over in the lipid-extracted microalgae biomass residues was successfully recovered for maltodextrin production (co-product). 90 % of maltodextrin yield could be attained by using 3 vol. % of H_2SO_4 (or 0.56 M) at operating temperature of 90^{o}C after 1 hour of hydrolysis time. Apart from that, pilot-scale cultivation of *Chlorella vulgaris* in a 100 L sequential baffled photobioreactor was carried out in the present study. The highest biomass yield attained under indoor and outdoor environment was 0.52 g/L and 0.28 g/L, respectively. Although low microalgae biomass yield was attained under outdoor cultivation, however, the overall life cycle energy efficiency ratio was 3.3 times higher than the indoor cultivation. It was found that negative energy balance was observed in producing the microalgae biodiesel for both indoor and outdoor cultivation. The minimum microalgae biodiesel production cost was about RM 237/L, which was exceptionally high compared to the current petrol diesel price (RM 3.6/L). On the other hand, the estimated production cost of dried microalgae biomass was RM 46/kg, which was lower than cultivation using chemical fertilizer (RM 111/kg) and current market price of *Chlorella* biomass (RM 145/kg). The growth of *Chlorella vulgaris* in the present study was found to fit well with the Richards model, with the highest R^2 value and displayed the lowest RMSD and variance values.

CHAPTER ONE:

INTRODUCTION

1.1 Current status of fossil fuel and renewable energy

Since the last few decades, fossil fuels have become an integral part of human daily lives. Specifically, fossil fuels are burned to produce energy for transportation and electricity generation, in which these two sectors have played a vital role in improving human living standard and accelerating advance technological development. In 2010, fossil fuels accounted for about 81 % (or 12,717 million tonne of oil equivalent) of the world's primary energy use, in which crude petroleum oil, coal and natural gas contributed 32.4 %, 27.3 % and 21.4 %, respectively, to this total energy supply (International Energy Agency, 2012). Specifically, global consumption of fossil diesel fuel was estimated to be 934 million tonnes per year (Kulkarni and Dalai, 2006).

Thus, there is no doubt that fossil fuels will be exhausted in less than 10 decades as predicted by The World Energy Forum if no new oil well is found (Sharma and Singh, 2009). The concern regarding the stingy crunch of energy resources is caused by rapid growth in human population, industrialization and urbanization (Huang and Wang, 2013). Hence, the era of inexpensive fossil fuel no longer exists; instead, the world is facing a shortage in the fossil fuel supply, bitter conflicts, and an increasing number of undernourished people, especially in the undeveloped countries (Lam et al., 2010).

Furthermore, burning fossil fuels have raised numerous environmental concerns, including greenhouse gas (GHG) emission which is the main cause of global warming. In the recent years, the impacts of global warming have caused severe damages towards human and environment ecosystem, such as melting of arctic ice that reduces the natural habitat of polar bears, rising of sea level resulted to inundation of low-lying islands, warmer water causing massive dying of sea coral, extreme heat waves continue to hamper agricultural sector and affecting human's health and frequent occurrence of droughts and desertification (Ho et al., 2011, Huang and Wang, 2013). The consequences of all these phenomena combined with the rising prices of energy have raised the public awareness to reduce fossil fuels consumption and to lower their personal shares in GHG emission (Yang et al., 2012).

One of the potential solutions to this problem is the continuous development of renewable and sustainable energy sector for the benefits of human and environment. **Figure 1.1** shows the projection of energy demand by sector indicating that there is an urgent need to find more new renewable energy sources to overcome the global energy crisis and for the benefits of human and environment (Exxon Mobil, 2013). Renewable energy sources such as solar energy, wind energy, hydro energy, and energy from biomass and waste have been successfully developed and used by different nations to limit the use of fossil fuels.

Nevertheless, based on recent study by International Energy Agency (IEA), only energy produced from biofuels and waste has the highest potential among other renewable resources (International Energy Agency, 2012). From the report, biofuels and waste accounted for 10.0% of the total energy supply, compared to hydro energy 2.3 % and other 0.9 % (geothermal, solar, wind and heat). Hence, it was predicted that renewable energy from combustible sources such as biodiesel will play a more crucial role as an alternative renewable fuel in the near future to further diversify the global energy sources.

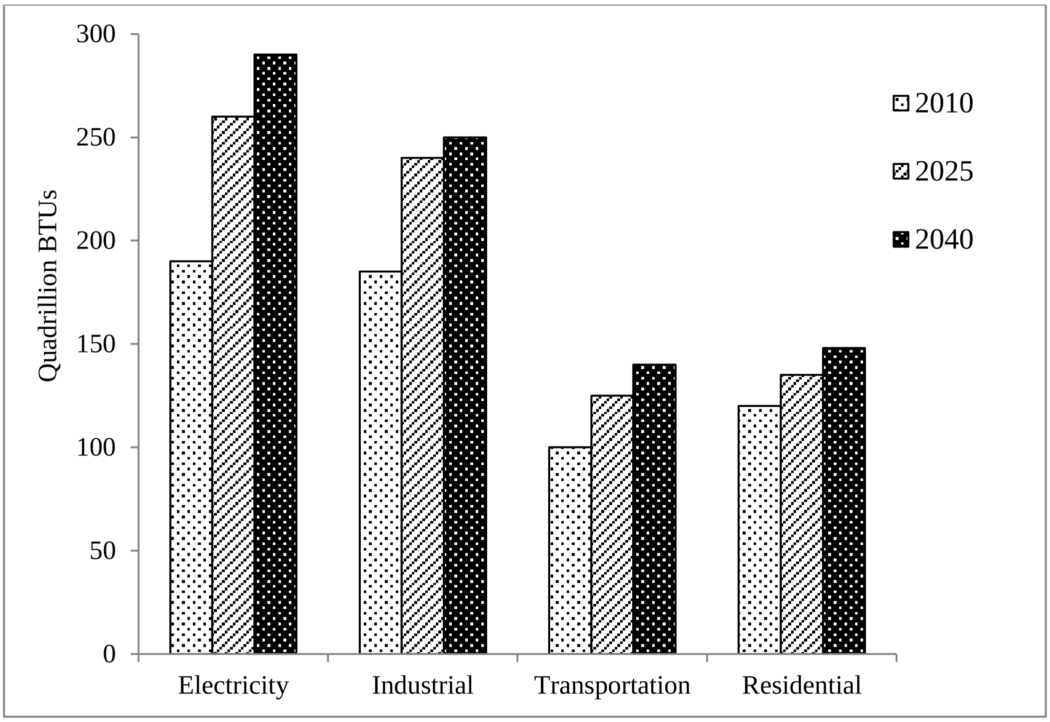


Figure 1.1: Projection of energy demand for the near future (Exxon Mobil, 2013)

1.2 Introduction to biodiesel

Biodiesel is a renewable diesel fuel, mainly derived from triglycerides sources such as vegetable oils, greases and animal fats (Vasudevan and Briggs, 2008). Triglycerides from these sources usually consist of different fatty acids, in which the composition of these fatty acids will be the most important factor influencing the corresponding properties of the produced biodiesel (Ramos et al., 2009). Fatty acids vary in terms of carbon chain length and number of unsaturated bonds (double bonds). For example, fatty acids that have no double bonds are termed "saturated" such as stearic acid. The carbon chains for these fatty acids contain maximum number of possible hydrogen atoms per carbon atom.

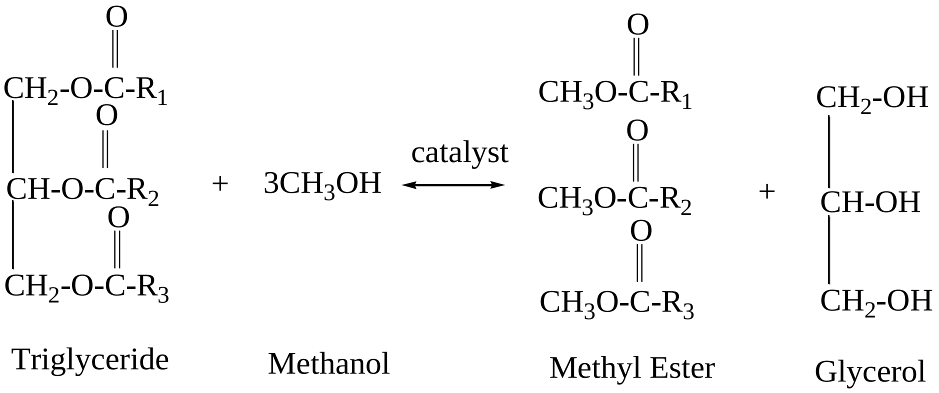
On the other hand, fatty acids that have double bonds are termed "unsaturated" such as linoleic acid. These fatty acids carbon chains do not contain maximum number of hydrogen atoms due to the presence of double bond(s) on some carbon atoms. **Table 1.1** summarized several common fatty acids found in edible and non-edible oils.

Table 1.1: Common fatty acid composition for different oil sources (Ma and Hanna, 1999, Balat and Balat, 2010, Yee et al., 2011, Kansedo and Lee, 2012)

Fatty acid	Edible Oil			Non-edible oil		
	Soybean	Rapeseed	Palm	Jatropha	Sea Mango	Microalgae
Lauric (C12:0)	0.1	-	0.1	-	-	-
Myristic (C14:0)	0.1	0.1	1	-	-	2.7
Palmitic (C16:0)	0.2	4.8	42.8	14.4	24.9	20.9
Palmitoleic (C16:1)	-	0.2	-	0.1	-	10.6
Stearic (C18:0)	3.7	1.9	4.5	3.6	5.8	6.9
Oleic (C18:1)	22.8	61.9	40.5	43.2	53	33.3
Linoleic (C18:2)	53.7	19.8	10.1	-	13.7	18.5
Linolenic (C18:3)	8.6	9.2	0.2	38.7	0.1	1.2

Direct use of vegetable oils and animal fats as combustible fuel is not suitable due to their high kinematic viscosity (about 11-17 times higher than diesel fuel) and low volatility (Meher et al., 2006, Mondal et al., 2008). Due to incomplete combustion and non-suitable vaporization characteristics of straight vegetable oils and animal fats, this will caused several severe problems to ignition diesel engine. This include coking and trumpet formation on the injectors to such an extent that fuel combustion does not occur, oil ring sticking and gelling of lubricating oil due to contamination of straight vegetable oils and animal fats (Muniyappa et al., 1996, Mondal et al., 2008). Consequently, the performance of diesel engine decreases and resulting to higher exhaust gas emissions of CO, NO_x and hydrocarbon. Thus, vegetable oils and animal fats must be subjected to chemical reaction such as transesterification to reduce the viscosity of the oils and to avoid its negative effect on the diesel engine during combustion.

In transesterification reaction, triglycerides are converted into fatty acid alkyl esters (biodiesel), in the presence of short chain alcohol, such as methanol or ethanol, and a catalyst, such as alkali or acid, with glycerol as a by-product (Vasudevan and Briggs, 2008). In the case when methanol is used as reactant, it will be a mixture of fatty acid methyl esters (FAME) whereas if ethanol is used as reactant, the mixture will be fatty acid ethyl esters (FAEE). Methanol is preferred to be used in biodiesel production due to its low cost, widely available in the market and faster reaction rate than ethanol (Lam and Lee, 2011). **Equation 1.1** shows a typical transesterification reaction involving methanol as reactant. Another alternative way to produce biodiesel is through thermal cracking or pyrolysis. However, this process is rather complicated to operate and produce side products that have no commercial value (Sharma and Singh, 2009).



$$\begin{array}{l} CH_2\text{-}O\text{-}\overset{\overset{O}{\|}}{C}\text{-}R_1 \\ | \\ CH\text{-}O\text{-}\overset{\overset{O}{\|}}{C}\text{-}R_2 \\ | \\ CH_2\text{-}O\text{-}\overset{\overset{O}{\|}}{C}\text{-}R_3 \end{array} + 3CH_3OH \xleftrightarrow{\text{catalyst}} \begin{array}{l} CH_3O\text{-}\overset{\overset{O}{\|}}{C}\text{-}R_1 \\ CH_3O\text{-}\overset{\overset{O}{\|}}{C}\text{-}R_2 \\ CH_3O\text{-}\overset{\overset{O}{\|}}{C}\text{-}R_3 \end{array} + \begin{array}{l} CH_2\text{-}OH \\ | \\ CH\text{-}OH \\ | \\ CH_2\text{-}OH \end{array} \qquad (1.1)$$

1.3 Current status of biodiesel production

With the crude fossil fuel price near all-time high, biodiesel has emerged as the fastest growing industries worldwide. Several countries especially United States of America (USA) and members of European Union (EU) are actively supporting the production of biodiesel from the agriculture sector. The progress of biodiesel production can be clearly seen in **Figure 1.2** (Hervé et al., 2011). In year 2000, the world production of biodiesel was merely 0.8 billion liters. The total biodiesel production reaches 4 billion liters after 5 years and more than 16 billion liters ten years later (Hervé et al., 2011).

EU countries are the major producer of biodiesel, accounted for 55 % of the market share in year 2010. This is due to substantial support from government such as consumption incentive (fuel tax reduction) and production incentive (tax incentives and loan guarantees) that has and will further accelerate the global market of biodiesel to grow explosively in the next ten years. Other non-EU countries such as Argentina, Brazil and USA are also experiencing an increase in biodiesel production. However, for US, the decreasing trend from 2008 to 2010 is due to the anti-dumping policy that imposed by the EU countries on US exports of biodiesel.

The total biodiesel production from the non-EU counties are 0.16 billion liters in year 2004 and increased to 7.7 billion liters in year 2009. By the year 2020, it is expected that biodiesel production from Brazil, China, India and some Asian countries such as Malaysia, Indonesia and Thailand could contribute as much as 20 % of the total biodiesel production (Multi-Client Study, 2008). The driving forces for development of biodiesel in these countries are economic, energy and environmental security, improving trade balances and expansion of agriculture sector (Zhou and

Thomson, 2009). If governments from these countries continue to aggressively promote biodiesel production and continue to invest in research and development for non-edible feedstock such as jatropha, castor and microalgae, the prospects to achieve biodiesel targets will be realized faster than anticipated.

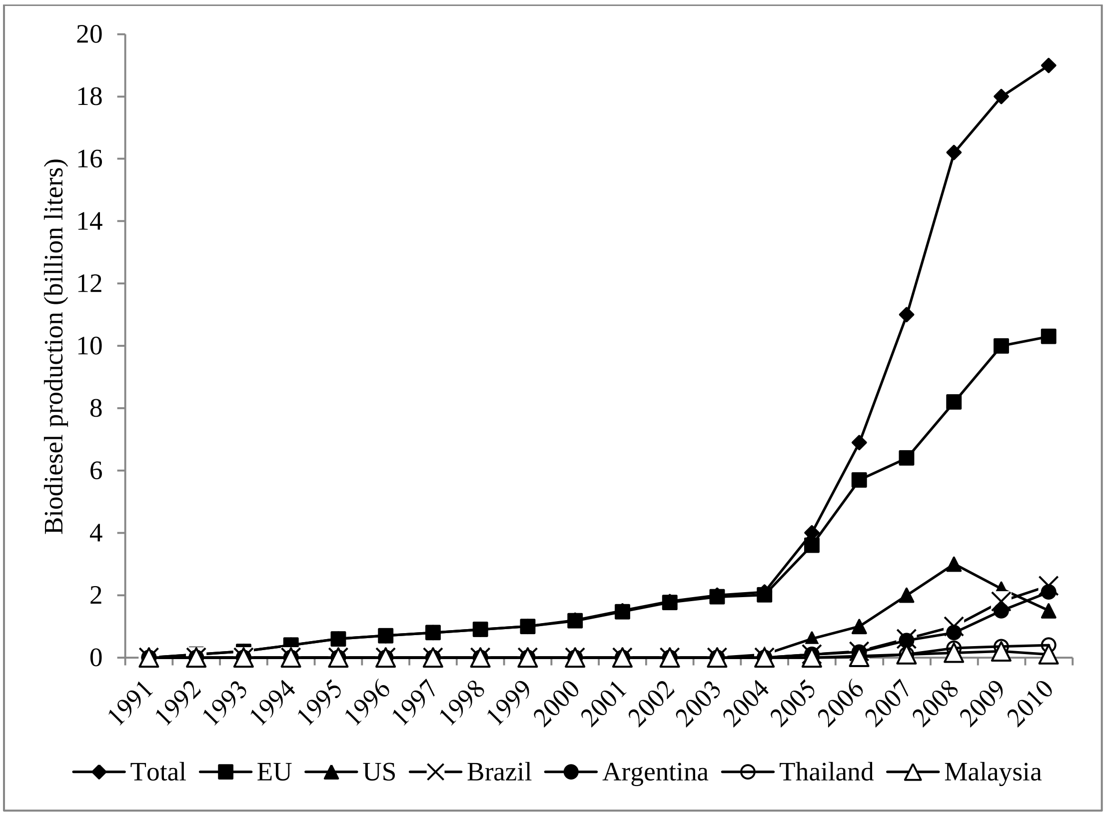


Figure 1.2: Biodiesel production from main producing countries, 1991-2010 (Hervé et al., 2011)

1.4 First and second generation biodiesel

First-generation biodiesel which has attained commercial-scale production in several countries is generally produced from edible oils using conventional technology (Singh et al., 2011b). The edible oils include soybean, rapeseed, palm and sunflower. Normally, the crude edible oil is extracted through mechanical pressing and refined before diverting to biodiesel production process. However, the viability and sustainability of the first-generation biodiesel are questionable, mainly due to the food versus fuel feud, low oil yield that resulted to larger arable land is required to accommodate the increasing oil demand, heavy fertilization, huge water requirement, and issue related to biodiversity conservation (Mata et al., 2010, Singh et al., 2011a, Singh et al., 2011b). This will certainly raises the price of food-grade oils in the global market, causing the production cost of biodiesel to increase and slowly losing its competitive advantages compared to fossil diesel (Lin et al., 2011). In fact, the cost of edible oils contribute nearly 80 % of the overall biodiesel production cost; an important factor that determines its commercial value and economic feasibility (Lam et al., 2009b).

Based on the current edible oils production rate, it is still in the infancy stage to fulfill the EU’s target on the 10 % market share of biodiesel by year 2020 due to the limited arable land for bio-energy crops (Mata et al., 2010). This can be clearly seen in **Figure 1.3** which shows that although the overall edible oil production is increasing, the ending stocks of the oil as food feedstock are continuously decreasing due to the expansion of biodiesel (Gui et al., 2008). As a result, one day, the edible oil supply may not be enough to fulfill its demand as food source if new renewable oil source is not explored for biodiesel production.

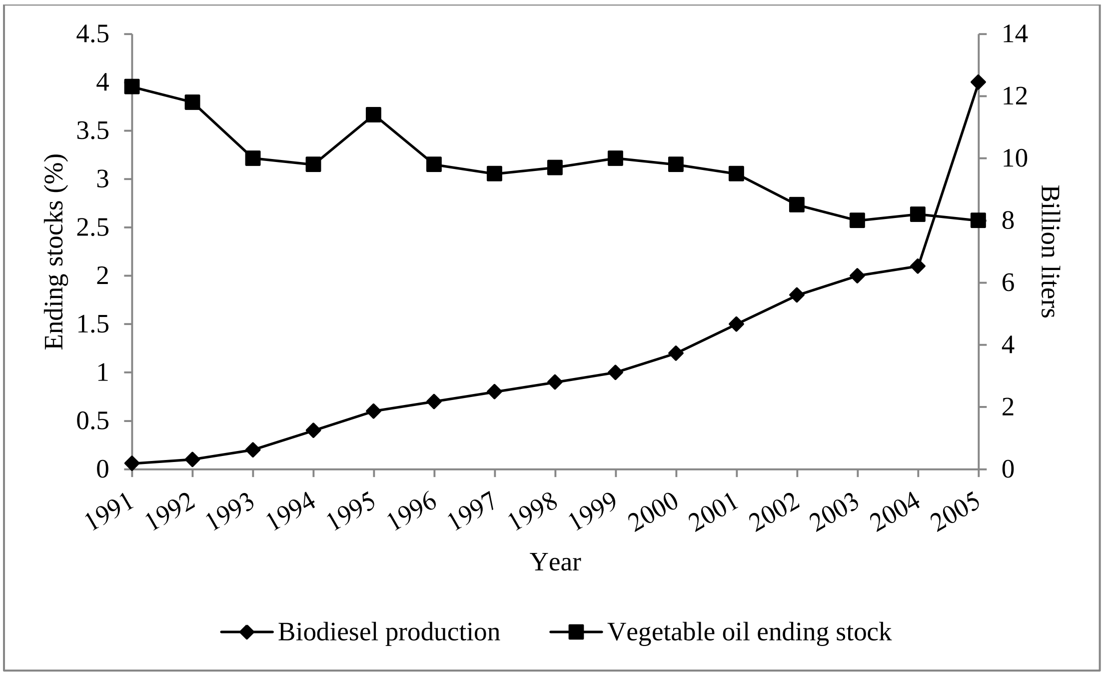


Figure 1.3: Vegetable oil ending stocks and biodiesel production (Gui et al., 2008, Hervé et al., 2011)

Hence, second-generation biodiesel derived from non-edible oils, such as *Jatropha curcas* L., *Cerbera odollam* (sea mango), *Moringa oleifera* and *Karanja* appeared as an attractive alternative feedstock (Kumar and Sharma, 2008, Kansedo et al., 2009, Kafuku et al., 2010, Lam et al., 2010, Borugadda and Goud, 2012). These non-edible oils usually contain high concentration of toxic compounds which are not suitable for human consumption or as nutrition supplement. For example, the toxic compound found in *Jatropha* oil is protein crucin, gluosidase cerberin in *Cerbera odollam* oil and flavonoids pongamiin and karajiin in *Karanja* oil (Gui et al., 2008, Banković-Ilić et al., 2012).

Among all the non-edible oils sources, *Jatropha* oil is the most promising and widely accepted feedstock for biodiesel production. *Jatropha* is a drought-resistant plant which is widely distributed in the wild or semi-cultivated in areas of Central and South America, Africa, India and South East Asia (Lam et al., 2009a). The average oil content in the dry *Jatropha* seed is about 34.4% (mass basis) (Achten et al., 2008).

To date, *Jatropha* oil is the main feedstock for biodiesel production in China, with estimated production rate of 170,000 tonnes annually (Yang et al., 2012). Although *Jatropha* plant can be grown on wasteland or non-fertile soil, however the overall seed yield is only 2.38 tonne/hectare/year; instead of 12 tonne/hectare/year when the plant is grown on fertile land (Achten et al., 2008, Lam et al., 2009a). Thus, regular irrigation, heavy fertilization and good management practises are still required to ensure a high seed yield from *Jatropha* plant.

1.5 An outlook of microalgae biomass as the third generation biodiesel

1.5.1 Introduction to microalgae

Microalgae are one of the oldest living microorganisms on Earth (Song et al., 2008). They are single cell organisms, representative of both bacteria and eukaryotes. A significant characteristic that distinguish between bacteria and eukaryotes is that the former lack of discrete internal, sub-cellular structures, organelles (chloroplasts, mitochondria and nuclei) (Williams and Laurens, 2010). Eukaryotes, which comprise of many different types of common microalgae, do have organelles that control the functions of the cell, allowing it to survive and reproduce (Brennan and Owende, 2010). To date, microalgae species are divided into four categories: diatoms (Bacillariophyceae), green algae (Chlorophyceae), blue-green algae (Cyanophyceae) and golden algae (Chrysophyceae), depending on their pigmentation, life cycle and basic cellular structure (Khan et al., 2009). Carbohydrates, proteins, nucleic acids and lipids are the major constituents of microalgae (Williams and Laurens, 2010).

1.5.2 Advantages of microalgae biodiesel

In the last few years, research on growing microalgae for biofuels production has gained increasing attention from various research groups across the world. Researchers have demonstrated the potential of converting lipid and carbohydrate from microalgae biomass to biodiesel and bioethanol, respectively, which are alternative fuels to existing fossil diesel and gasoline.

One of the reasons that microalgae appear as an attractive renewable energy source is due to its rapid growth rate; 100 times faster than land-based plant and they can double their biomass in less than 1 day (Tredici, 2010). Furthermore, microalgae are able to divide once every 3–4 hours, but mostly divide every 1–2 days under favourable growing conditions (Williams and Laurens, 2010). This is mainly due to their simple cellular structure and large surface to volume ratio that allow them to uptake large amount of nutrients from water sources (Khan et al., 2009).

Apart from that, microalgae can be cultivated either phototrophic or heterotrophic. Phototrophic microalgae such as *Botryococcus braunii* and *Dunaliella salina* require sunlight, CO_2 and nutrients as a basic requirement for growing whereas heterotrophic microalgae such as *Chlorella protothecoides* require organic carbons sources (sugar and organic acids) and nutrients but do not require sunlight (Liang et al., 2009). In some special cases, for example *Cholorela protothecoides* can be grown phototrophically or heterotrophically under different cultivation conditions. However, heterotrophically growth of *Chlorella protothecoides* is more favourable due to higher accumulation of lipid content in cells (Miao and Wu, 2006).

The potential of microalgae cultivation for biofuels production can be clearly seen in **Table 1.2**. From the table, cultivating microalgae (either high or low lipid content) requires the least land area than other oil-bearing crops such as soybean, sunflower, rapeseed and oil palm in order to meet EU biofuels target in year 2010. According to recent studies, a realistic microalgae biomass production rate should lies between 15 and 25 tonne/ha/year. With an assumption of 30% lipid content in microalgae cells (without optimizing the growth condition), the microalgae lipid production rate is equivalent to 4.5–7.5 tonne/ha/year. This amount is certainly higher compared to the oil production from soybean (0.4 tonne/ha/year), rapeseed (0.68 tonne/ha/year), oil palm (3.62 tonne/ha/year) and jatropha (4.14 tonne/ha/year). An added advantage to microalgae biofuels is it does not compete land area with food production and thus, holding an important key for a sustainable energy development in the future.

Table 1.2. Comparison of oil yield for various oil bearing plants and microalgae (Chisti, 2007, Lam et al., 2009a, Lam et al., 2009b)

Oil crop	Average oil yield (tonne/ha/year)	Area to meet EU biodiesel demand in 2010 (million hectares)[a]	% of current Malaysian's agricultural land area[b]
Soybean	0.4	25.0	379
Sunflower	0.46	21.7	329
Rapeseed	0.68	14.7	223
Oil palm	3.62	2.8	42
Jatropha[c]	0.14	71.4	1082
Jatropha[d]	4.13	2.4	37
Microalgae[e]	126	0.1	1
Microalgae[f]	54	0.2	3

Note:

[a] EU biodiesel target in year 2010 is equivalent to 10 million tonnes

[b] Total agricultural land area in Malaysia is equivalent to 6.6 million hectares

[c] Jatropha are planted without irrigation and fertilization

[d] Jatropha are planted with irrigation and heavy fertilization

[e] Microalgae synthesized high lipid content (70 % lipid based on biomass weight)

[f] Microalgae synthesized low lipid content (30 % lipid based on biomass weight)

1.5.3 CO_2 mitigation and co-product production from microalgae biomass

Due to the advantages of fast growth rate and high lipid productivity, phototrophic microalgae can convert solar energy to chemical energy with efficiency of 10–50 times greater than terrestrial plants by fixing CO_2 from atmosphere, flue gases or soluble carbonate during photosynthesis (Li et al., 2008b, Khan et al., 2009, Rosenberg et al., 2011). Furthermore, it was reported that microalgae cells contain approximately 50% carbon, in which 1.8 kg of CO_2 are fixed by producing 1 kg of microalgae biomass; a golden opportunity for carbon credit program (Chisti, 2007). In addition, some microalgae strains have high adaptability and could withstand high concentration of CO_2 (up to 20 %), such as *Chlorella* sp., *Scenedesmus* sp. and *Botryococcus braunii* (Brennan and Owende, 2010, Yoo et al., 2010). Hence, this method is thought to be more technologically feasible and microalgae can act as an effective carbon sink in bio-fixing the CO_2 from atmosphere and flue gases while producing renewable green fuel.

Besides lipid, microalgae cells also consist of a large portion of carbohydrate which has high commercial value. Identified microalgae strains that have high carbohydrates content are such as *Chlamydomonas reinhardtii* (53 %), *C. reinhardtii* (45 %), *Chlorella vulgaris* (12-37 %), *Chlorella* sp. (21-27 %) and *Scenedesmus* sp. (13-20 %) (John et al., 2011). Different from terrestrial plants, microalgae cells are buoyant and do not require lignin and hemicelluloses for structural support. Therefore, it is expected that carbohydrate extraction from microalgae biomass are simpler than lignocellulosic materials (e.g. wood), in which complicated pre-treatment steps to remove the lignin can be avoided. In fact, some of the pre-treatment methods, such as ozonolysis, organosolv, steam explosion and ammonia fiber explosion (AFEX) are usually costly and generate toxic compounds to the environment if proper waste treatment system is not implemented (Cardona and Sánchez, 2007, Alvira et al., 2010). After lipid extraction, the carbohydrate remaining in the microalgae residues can be further hydrolyzed to simple reducing sugar (e.g. glucose) for subsequent fermentation process to produce bioethanol, which is an alternative renewable fuel to gasoline. Besides being used for fermentation, the hydrolyzed carbohydrate has a wide range of industrial applications, such as water soluble glues, thickening agents in food processing, and binding agents in the pharmaceutical industry (Biswas et al., 2009).

1.6 Problem Statement

In the last few years, researches on growing microalgae for biofuel production have gained increasing attention from various research groups across the world. Researchers have demonstrated the potential of converting lipid and carbohydrate from microalgae biomass to biodiesel and bioethanol, respectively, which are alternative fuels to existing fossil diesel and gasoline. However, several recent life cycle assessments (LCA) on microalgae biofuels have demonstrated that massive energy input are required in producing the biofuels, especially during the cultivation and harvesting of microalgae biomass. One of the limitations to cultivate microalgae at industrial scale is the availability of low cost nutrients sources. Chemical or inorganic fertilizers are commonly used to achieve promising growth rate of microalgae are relatively expensive and not environmentally friendly for long term usage. On the other hand, utilizing secondary or tertiary wastewater as nutrients source to cultivate microalgae appears as a promising choice to reduce the overall energy input. Nevertheless, the key challenges of using wastewater as cultivation medium are serious contamination and inconsistent nutrients composition, in which these factors will significantly retard the growth of microalgae. Other associated problems that directly impede the commercialization of microalgae biodiesel are such as possibility of outdoor cultivation, efficiency of CO_2 capture by microalgae, issue of life cycle energy balance and economic feasibility.

1.7 Objectives

Current research work focused on the following objectives:

1. To optimize the growing conditions of *Chlorella vulgaris* using organic fertilizer as nutrient source and to study the effect of carbon source towards the growth and lipid accumulation in microalgae.
2. To extract the lipid from dried microalgae biomass and to optimize the transesterification of lipid to biodiesel.
3. To optimize the carbohydrate hydrolysis condition from lipid-extracted microalgae biomass residue for maltodextrin production.
4. To scale up the microalgae cultivation in a vertical column photobioreactor (pilot scale).
5. To evaluate the life cycle energy balance and economic assessment of microalgae biodiesel production.
6. To evaluate the growth kinetic of *Chlorella vulgaris* when cultivated using organic fertilizer as nutrients source.

1.8 Scope of study

1.8.1 Microalgae cultivation

Optimization on the growth of *Chlorella vulgaris* using organic fertilizer as nutrient source was performed. *Chlorella vulgaris* was selected in the present study because it is easy to cultivate, able to grow under contaminated environment and is a native species in Malaysia. Two cultivation methods were assessed, which were free cells cultivation and immobilization cultivation. Growth parameters, such as amount of nutrients, cultivation pH, light exposure duration and effect of outdoor cultivation were studied. The growth performance of the microalgae was evaluated based on their specific growth rate, biomass yield and biomass productivity. Since the aim of the present study is to optimize the microalgae biomass productivity, the effect of limited nitrogen source (which was reported to be able to increase the lipid content in microalgae cells, but with lower biomass productivity) was excluded in the study.

The effect of different carbon sources towards the growth of the microalgae was also studied. Two types of carbon source, namely CO_2 gas and sodium bicarbonate, were used as the carbon source to cultivate *Chlorella vulgaris*. Different concentration of CO_2 gas or sodium bicarbonate was varied to optimize the growth of microalgae. The result was tabulated in term of carbon removal efficiency, specific growth rate, biomass yield and biomass productivity. Since the present study focused on phototrophic cultivation, the effect of other carbon source, such as glucose, glycerol and volatile fatty acids, were not assessed because the carbon source will only be utilized by microalgae in the absence of light (heterotrophic cultivation).

1.8.2 Lipid extraction and transesterification

Dried microalgae biomass was subjected to lipid extraction using various chemical solvents, such as hexane, methanol, ethanol and chloroform. The performance of the chemical solvents was determined based on the lipid yield obtained. Then, the extracted microalgae lipid was converted to biodiesel through transesterification. Various reaction parameters will be assessed to optimize the microalgae biodiesel conversion, such as reaction temperature, methanol to lipid molar ratio, catalyst concentration and effect of co-solvents. Only homogeneous acid catalyst (H_2SO_4) was utilized in the present study due to the high free fatty acid (FFA) content in the microalgae lipid. Heterogeneous acid catalyst was not included in this work since the catalyst are mostly still at research and development stage and yet to be commercialized.

1.8.3 Co-product production

The lipid-extracted microalgae residues were utilized for maltodextrin production by hydrolyzing the carbohydrate. Various hydrolysis parameters were assessed to optimize the maltodextrin yield, such as hydrolysis reagents (acid, alkaline and enzymatic), hydrolysis temperature and duration. However, in this study the microalgae residue was not subjected to protein extraction as co-product since the *Chlorella vulgaris* was cultivated under contaminated conditions. The extracted protein (usually used for human and animal consumption) may require extensive purification that will indirectly impede sustainable production of microalgae biodiesel through bio-refinery concept.

1.8.4 Photobioreactor design and scale up study

A pilot-scale column photobioreactor with working volume of 100 L was designed based on the lab-scale experimental results. The lab-scale optimum cultivation conditions were applied in the pilot-scale photobioreactor to validate the reproducibility of the result. Potential of semi-batch cultivation under indoor and outdoor environment in the pilot-scale photobioreactor was also carried out to accelerate the biomass productivity.

1.8.5 Life cycle energy balance analysis and economic assessment

Life cycle energy balance on producing microalgae biodiesel was performed based on the experimental data obtained in the present study. The life cycle boundary includes cultivation of *Chlorella vulgaris*, harvesting and drying of microalgae biomass, lipid extraction, biodiesel and maltodextrin production. Energy efficiency ratio (EER) was used as an indicator to determine the sustainability of microalgae biodiesel production from the energy perspective. Apart from that, economic assessment on producing the *Chlorella vulgaris* biomass and biodiesel was also carried out in the present study to estimate the economic potential of this renewable feedstock. However, the capital cost (cost of land, buildings, equipment and infrastructures) was excluded in the assessment due to limited information available.

1.8.6 Growth kinetic of microalgae

The growth kinetic of *Chlorella vulgaris* was evaluated based on five non-linear mathematical models, namely logistic, Gompertz, modified Gompertz, Baranyi and Richards model. A non-linear regression technique was used to solve growth models by using POLYMATH 6.0.

1.9 Organization of thesis

This thesis consists of five chapters:

Chapter one gives an outline of the overall research project covering introduction to biodiesel, current status of biodiesel market and potential of microalgae biomass as the third generation feedstock for biodiesel production. Problem statement was then written after reviewing the present scenario and related issues in producing microalgae biodiesel. The problem statement therefore reveals current bottlenecks faced in the bio-refinery of microalgae biodiesel and the need of this research project. The objectives of this research project were then carefully devised with the aim to improve the sustainability of microalgae biodiesel and increase its potential for commercialization purposes. Finally, the organization of thesis highlights the content of each chapter.

Chapter two gives an overall review of microalgae cultivation for biofuel production. The review started with the bio-refinery concept of microalgae biofuel, advantages of microalgae biomass compared with other renewable sources and the opportunity of CO_2 bio-mitigation by microalgae. Then, related problems and issues facing the microalgae biofuels production were critically depicted through the latest findings from LCA. Apart from that, technical information on the entire microalgae biodiesel process chain, ranging from upstream (microalgae cultivation, biomass harvesting and drying) to downstream processes (lipid extraction and biodiesel conversion techniques) are also included in this chapter.

Chapter three mainly discusses on experimental materials and research methodology. This chapter describes detailed information on the flow of this research work, starting from seed cultivation of microalgae until biodiesel and maltodextrin production. Besides, information on the chemicals used in this study as well as several analytical methods and tools were also being described. Kinetic study and modelling on the growth of microalgae was included at the last part of this chapter.

Chapter four is the most important chapter in the thesis. It encompasses detailed discussion on the results obtained in the present research work. The first section discussed the optimization result of using the organic fertilizer as the nutrients source to cultivate *Chlorella vulgaris* via either free cell cultivation or immobilization cultivation. Then, the effect of carbon sources (CO_2 and bicarbonate) towards the growth of microalgae was carefully evaluated. This was followed by study on lipid extraction from dried microalgae biomass and optimization of microalgae lipid conversion to biodiesel through catalytic transesterification. Section four discussed the potential utilization of lipid extracted microalgae residue for maltodextrin production whereas section five discussed the scale up study (pilot scale) of *Chlorella vulgaris* in a vertical column photobioreactor. LCA of producing microalgae biodiesel in this particular study was revealed in section six. At the end of this chapter, kinetic study and modelling on the growth of *Chlorella vulgaris* was presented.

Chapter five is the last chapter in this thesis that gives concluding remarks of all the findings in this research work and recommendations for future study.

CHAPTER TWO:

LITERATURE REVIEW

This chapter reviews studies that are related to microalgae cultivation and biodiesel production from this renewable feedstock. This chapter focused on the findings from the life cycle energy balance in the process flow of producing microalgae biodiesel and to identify the actual problems and research that are required to improve the processes. Then, critical reviews and comments on each of the process flow will be provided as a platform to facilitate a better understanding on the actual issues, sustainability and prospective of microalgae biodiesel. The process flow includes the nutrients source, cultivation system, harvesting and drying of microalgae biomass, additional CO_2 supplement, lipid extraction, microalgae biodiesel production methods and potential utilization of lipid-extracted microalgae residue. A summary will be provided at the end of this chapter to outline some important notes on the overall process flow of microalgae biodiesel production.

2.1 Life cycle assessment (LCA) of microalgae biofuels

Although microalgae biofuels (mainly refer to biodiesel and bioethanol) have been predicted to make a significant contribution in diversifying the global renewable energy sector, however, the long term sustainability of this renewable feedstock is still questionable. Up to now, there is still no commercial plant producing and processing microalgae biomass into biofuels. This has subsequently caused a lack of understanding in each of the unit operations in the entire process at industrial scale. A conceptual process flow of producing microalgae biodiesel and other related co-products is shown in **Figure 2.1**. The process chain can be divided into two sections: (1) up-stream process which includes microalgae cultivation system, harvesting and drying of microalgae biomass, and (2) downstream process which focuses on biodiesel production and utilization of microalgae biomass residue for bioethanol production.

LCA is widely accepted as an effective tool to guide and give a clear idea to researchers and policy makers on revealing the real potential of a particular product that is being evaluated (Lam et al., 2009a). It also can be used to indicate if the production of a particular product can lead to negative environmental phenomena such as eutrophication, global warming, ozone depletion, human and marine toxicity, land competition, photochemical oxidation and etc. so that precautionary steps can be suggested to reduce the negative impacts (Andersson, 2000). In addition, energy balance can be calculated to determine and justify the energy hotspot of all stages within the system boundary of the LCA.

Apparently, there are only a few LCAs performed on microalgae biofuels due to limited comprehensive data. Therefore, parameters related to microalgae biofuels production such as biomass productivity, lipid content and downstream energy consumption (harvesting, drying and transesterification) were obtained based purely on lab scale experimental data. Although the data used in those assessments might be irrelevant when applied to large-scale production, however, most of the studies have concluded that producing biofuels from microalgae is an extremely energy intensive process. This finding is represented by the energy efficiency ratio (EER), defined as energy output to energy input, which is generally used to indicate the sustainability energy index to produce a particular product, in which a ratio higher than 1 designates to net positive energy generated and vice versa.

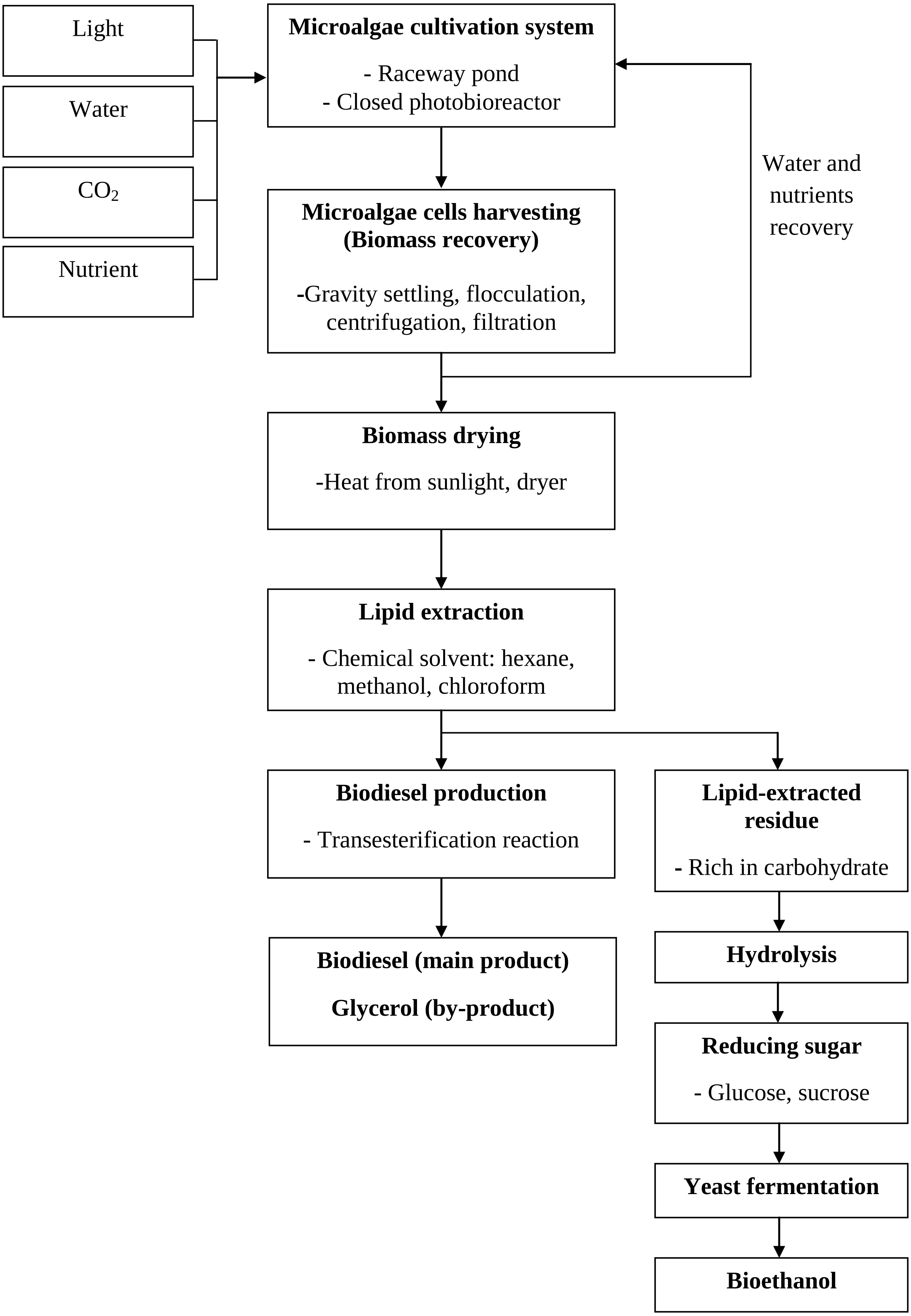


Figure 2.1: Process flow of producing microalgae biodiesel and co-products