

**NEW MICROWAVE BASED TRANSESTERIFICATION TECHNIQUES FOR  
BIODIESEL PRODUCTION FROM CULTIVATED MICROALGAE**

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**A graceful dedication to my lovely family members,  
friends and honor supervisors**

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## ABSTRACT

Microalgae biodiesel production has its share of problems such as the high cost in microalgae productivity, harvesting, dewatering, lipid extraction and transesterification. Thus research in this field focuses on addressing these issues from the cultivation perspective and also the processing aspect. In this dissertation both approaches were used to address the problems where marine microalgae (*Tetraselmis* sp. and *Nannochloropsis* sp.) were cultivated indoor using light emitting diodes (LED) and processed using direct transesterification (DT). During cultivation, factors influencing microalgae growth rate and lipid content such as the type of LED wavelength and light intensities were investigated in detail. Microalgae were cultivated for 14 days as under blue, red, red-blue LED and white fluorescent light. The intensity of the red, blue and mixed red-blue LED was varied at 100, 150 and 200  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ . Findings revealed both species prefer to grow under blue wavelength which showed highest growth rate (reflected by the high cell count and absorbance readings) and lipid content (indicated by the fluorescence intensity). Suitable combination of LED wavelengths and intensity; (red LED: 150, blue: 100 and mixed red-blue: 200  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ ) produced maximum biomass growth and lipid content. Then, several techniques were used to improve the DT method; i) introducing the use of microwave heating (MWH) to the various extraction methods, ii) varying the type of catalyst under MWH and iii) the use of simultaneous cooling and microwave heating (SCMH). Furthermore, four different methods were used to extract the lipids: Hara and Radin, Folch, Chen and Bligh and Dyer. They were performed under MWH and conventional heating using water bath heating. Lipid yield for *Tetraselmis* sp. and *Nannochloropsis* sp. was highest when Hara and Radin (8.19%), and Folch (8.47%) methods were used respectively under MWH. *Nannochloropsis* sp. wet biomass was transesterified under MWH in the presence of methanol and various alkali and acid catalyst using two different types of DT; one step and two step transesterification. The biodiesel yield obtained from the microwave direct transesterification (MWDT) was compared with that obtained using conventional method (lipid extraction followed by transesterification) and water bath heating DT method. Findings revealed that MWDT efficiency was higher compared to water bath heating DT by at least 14.34% and can achieve a maximum of 43.37% with proper selection of catalysts. The use of combined catalyst (NaOH and H<sub>2</sub>SO<sub>4</sub>) increased the yield obtained by 2.3 folds (water bath heating DT) and 2.87 folds (MWDT) compared with the one step single alkaline catalyst. Maximum yield was obtained using SCMHW when the microwave was set at 50 °C, 800 W, 16 h of reaction with simultaneous cooling at 15 °C. When the one step transesterification was performed in SCMHW at optimum setting, the biodiesel yield was more than 3.75 folds than conventional method. Gas chromatography analysis depicted that the biodiesel produced from SCMHW had shorter carbon chain fatty acid methyl esters (<19 C) and good cetane number and iodine value indicating good ignition and lubricating properties. Thus it was proven that the use of LED, MWH and SCMHW can improve microalgae biodiesel yields.

## ABSTRAK

Penghasilan biodiesel daripada mikroalga mempunyai masalah seperti kos yang tinggi semasa pengkulturan mikroalga, penuaian, penyahairan, pengekstrakan lipid dan pentransesteran. Oleh itu, penyelidikan dalam bidang ini tertumpu untuk menangani masalah-masalah daripada aspek pengkulturan dan pemprosesan mikroalga. Dalam penyelidikan ini, *Tetraselmis* sp. dan *Nannochloropsis* sp. dikultur secara tertutup dengan diod pemancar cahaya (LED) dan diproses menggunakan pentransesteran langsung (DT). Semasa proses pengkulturan, faktor yang mempengaruhi kadar pengkulturan dan kandungan lipid seperti jenis panjang gelombang LED dan tahap keamatan cahaya disiasat secara terperinci. Mikroalga telah dikultur selama 14 hari di bawah cahaya biru, merah, merah-biru LED dan pendarfluor putih. Kesan keamatan cahaya dari cahaya merah, biru dan merah-biru yang diubah pada 100, 150 dan 200  $\mu\text{mol foton m}^{-2}\text{s}^{-1}$  telah disiasat. Keputusan menunjukkan mikroalga lebih memilih untuk tumbuh di bawah cahaya biru dengan memberi kadar pertumbuhan tertinggi (digambarkan oleh bilangan sel yang tinggi dan bacaan keserapan) dan kandungan lipid (ditunjukkan oleh tahap keamatan pendarfluor). Panjang gelombang LED dan paras keamatan yang sesuai; (LED merah:150, biru:100 dan merah-biru: 200  $\mu\text{mol foton m}^{-2}\text{s}^{-1}$ ) menghasilkan biojisim dan lipid tertinggi. Beberapa teknik telah digunakan untuk memperbaiki kaedah DT i) teknik pemanasan gelombang mikro (MWH) kepada pelbagai kaedah pengekstrakan, ii) penggunaan pemangkin yang berlainan menggunakan MWH iii) penggunaan penyejukan dan pemanasan gelombang mikro (SCMH) serentak. Di samping itu, empat kaedah yang berbeza telah digunakan untuk mengekstrak lipid: Hara dan Radin, Folch, Chen dan Bligh dan Dyer. Kaedah ini dilakukan dengan menggunakan MWH dan pemanasan konvensional menggunakan rendaman air. Penghasilan lipid paling tinggi bagi *Tetraselmis* sp. dan *Nannochloropsis* sp. adalah apabila kaedah Hara dan Radin (8.19%), dan Folch (8.47%) bersama MWH digunakan. Biojisim basah *Nannochloropsis* sp. melalui proses DT menggunakan MWH dengan kehadiran metanol dan pelbagai pemangkin asid dan alkali menggunakan dua jenis DT iaitu; satu langkah dan dua langkah. Penghasilan biodiesel dari kaedah pentransesteran langsung dengan pemanasan gelombang mikro (MWDT) dibandingkan dengan kaedah konvensional (pengekstrakan lipid diikuti pentransesteran) dan kaedah DT pemanasan rendaman air. Kajian menunjukkan kecekapan MWDT adalah lebih tinggi berbanding DT pemanasan rendaman air, iaitu 14.34% dan boleh mencapai kecekapan maksimum sehingga 43.37% jika menggunakan pemangkin yang sesuai. Penggunaan gabungan pemangkin (NaOH dan H<sub>2</sub>SO<sub>4</sub>) meningkatkan penghasilan biodiesel sehingga 2.3 kali ganda (pemanasan rendaman air DT) dan 2.87 kali ganda (MWDT) berbanding dengan pemangkin alkali satu langkah. Hasil maksimum diperolehi apabila SCMH ditetapkan pada 50 °C, 800 W, untuk tindak balas selama 16 jam dengan penyejukan serentak pada 15 °C. Apabila pentransesteran satu langkah dijalankan di dalam SCMH pada keadaan optimum, biodiesel yang terhasil adalah lebih daripada 3.75 kali ganda berbanding kaedah konvensional. Analisis gas kromatografi membuktikan bahawa biodiesel yang dihasilkan melalui SCMW mempunyai rantaian karbon yang pendek (<19 C), nombor setana dan nilai iodin yang baik menunjukkan ia mempunyai nyalaan dan sifat pelincir yang baik. Kajian ini membuktikan bahawa penggunaan LED, MWH dan SCMW dapat meningkatkan penghasilan biodiesel daripada mikroalga.

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**LIST OF ABBREVIATIONS**

ATP	-	Adenine triphosphate
C	-	Carbon
CH	-	Conventional heating
C <sub>3</sub> H <sub>7</sub> OH	-	Iso-propanol
C <sub>6</sub> H <sub>14</sub>	-	Hexane
CHCl <sub>3</sub>	-	Chloroform
CH <sub>3</sub> OH	-	Methanol
CN	-	Cetane number
DHA	-	Docosahexanoic acid
DT	-	Direct transesterification
DU	-	Degree of unsaturation
EPA	-	Eicosapentaenoic acid
FAME	-	Fatty acid methyl ester
FCC	-	Federal Communications Commission
FFAs	-	Free fatty acids
GC	-	Gas Chromatography
HCl	-	Hydrochloric acid
H <sub>2</sub> SO <sub>4</sub>	-	Sulphuric acid
IPPC	-	Intergovernmental Panel on Climate Change
IR	-	Infrared
IV	-	Iodine value
KCl	-	Potassium chloride
LED	-	Light emitting diode
LHC	-	Light harvesting complexes
MW	-	Microwave
MWDT	-	Microwave direct transesterification
MWH	-	Microwave heating
MWOST	-	Microwave one step transesterification

N	-	Nitrogen
NADP+	-	Nicotinamideadenine dinucleotide phosphate
NaH <sub>2</sub> PO <sub>4</sub> ·2H <sub>2</sub> O	-	Dihydrate sodium dihydrogen phosphate
NaNO <sub>3</sub>	-	Sodium nitrate
NaOH	-	Sodium hydroxide
OST	-	One step transesterification
P	-	Phosphorus
pH	-	Potential of Hydrogen
PUFAS	-	Polyunsaturated fatty acids
RM	-	Ringgit Malaysia
SC-IST/ E	-	supercritical <i>in situ</i> transesterification
SCMH	-	Simultaneous cooling and microwave heating
sp.	-	Species
TST	-	Two step transesterification
UHF	-	Ultra high frequency
US\$	-	United States Dollar
VHF	-	Very high frequency

## LIST OF SYMBOLS

%	-	Percent
<	-	Lower
°C	-	Degree Celcius
Cells ml <sup>-1</sup>	-	Cells per milliliter
DW	-	Dry weight
GHz	-	Giga hertz
g/L/ day	-	Gram per litre per day
g / m <sup>2</sup> / day	-	Gram per square meter per day
g DW/ L	-	Gram dry weight per litre
g DW/ L/ day	-	Gram dry weight per litre per day
g DW/ m <sup>2</sup> / day	-	Gram dry weight per square meter per day
g DW/ m <sup>2</sup> / h	-	Gram dry weight per square meter per hectare
H	-	Hour
kJ	-	Kilo joule
kW	-	Kilo watt
L	-	Litre
L m <sup>-2</sup> d <sup>-1</sup>	-	Litre per square meter per day
L/ha	-	Litre per hectare
Lux	-	Illuminance and luminous emittance
M ha	-	Mega hectare
M Hz	-	Mega hertz
M Pa	-	Mega Pascal
mg/ L	-	Milligram per litre
MHz	-	Megahertz
Min	-	Minute
ml	-	Milliliter
Mol	-	Mole
mmHg	-	Milliter of mercury
nm	-	Nanometre

rpm	-	Revolution per minute
Ton ha <sup>-1</sup> year <sup>-1</sup>	-	Tonne per hectare per year
TWh	-	TeraWatt-hour
μ mol photon m <sup>-2</sup> s <sup>-1</sup>	-	Micro mol photon per mili square per second
v/v	-	Volume per volume
W	-	Watt
W/ m <sup>2</sup>	-	Watt per meter square



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## CHAPTER 1

### INTRODUCTION

#### 1.1 Background of Research

Fossil fuel makes up 80% of the global energy demand. The vigorous use of fossil fuels has led to health problems, global climate change and environmental pollutions (Hallenbeck and Benemann, 2002). The depleting reserves of fossil fuel-based products have prompted scientists to search for renewable sources of energy (Shweta *et al.*, 2003). According to scientific sources such as, the Intergovernmental Panel on Climate Change (IPPC), the increased level of global warming is a man-made phenomenon and is contributing to the increased usage of fossil fuel. Therefore, vigorous research has been undertaken to seek alternative renewable biofuels as an alternative energy source with minimum adverse impact to the environment.

With the increasing need to decrease carbon production and the decreasing reserves of crude oil; liquid fuel derived from plant material also known as biofuel is an attractive substitute source of energy. Compared with other forms of renewable energy in the world such as wind, tidal and solar energy, biofuel allows energy to be chemically stored, and can also be used in existing engines and transportation infrastructures after combining with petrol or diesel in various proportions (Singh and Gu, 2010). Biodiesel has better lubricating property compared to today's lower viscosity diesel fuel (Chisti, 2007). Oil crops are renewable resources but biodiesel production from oil crops in huge amounts has been deemed economically unsustainable (Chisti, 2008). Production of crop derived biodiesel will need huge areas of arable land, which has to compete with the cultivation of food crops. This has led to the controversy of "food versus fuel" crisis (Searchinger *et al.*, 2008).

The increasing criticism of the unsustainable first generation biofuel from food crops such as sugarcane and corn has stimulated the interest in further developing second generation biofuel from feedstock such as lignocelluloses biomass (Yangmin and Mulan, 2011). The second generation biofuel include biodiesel produced from woody crops, agricultural waste or residues non food crop such as jatropha, waste cooking oil and animal fat (Spolaore *et al.*, 2006). Unfortunately, biofuel produced from non food crops such as jatropha also require large areas of cultivation land, which has to compete with the cultivation of food crops and thus has created currently a huge controversial issue (Chisti, 2007). Also, extraction of biodiesel from such biomass is more difficult due to the content of lignin.

Currently, there have been substantial research on biodiesel from microalgae (Pultz, 2001), which is considered as the third generation biofuel. Some regard microalgae as being the only renewable biofuel capable of meeting the whole world demand for transport fuel (Chisti, 2007). Amongst other attractive benefits, compared to traditional biofuel production based on crop, microalgae cultivation technology is well developed, does not compete with food production for arable land or water supply, is independent on soil fertility, and the annual production per unit area is much greater than those for crop (Williams, 2007). In fact, microalgae is gaining increased scientific interest due to its potential ability to produce and accumulate large quantities of neutral lipids (25%-50% of dry weight), normally in the form of triacylglycerol (Chen *et al.*, 2009), which is suitable for biofuel production.

Microalgae is considered as an alternative source of a wide range of chemicals, including highly valuable phycobiliproteins, carotenoids, antioxidants and long-chain polyunsaturated fatty acids (PUFAS) (Molina, 1995) and they play an important role in marine ecosystems (Feng *et al.*, 2012). Some genus of microalgae, with high growth rate and high lipid amount, appear to be attractive alternatives as resources for biodiesel production (Chisti, 2007; Hu *et al.*, 2008; Halim *et al.*, 2011). Microalgae are known as one of the oldest living microorganisms on Earth (Song *et al.*, 2008) and they grow at very fast rates; approximately one hundred times faster than terrestrial plants and their biomass are doubled in less than 24 hours (Tredici, 2011). Besides, some microalgae species are able to accumulate large amount of lipids inside their cells and the lipids can be transformed into biodiesel (Chisti, 2007). Previous research found that the marine microalgae *Nannochloropsis* sp. and *Tetraselmis suecica* have the potential to be the source of renewable oil (Rodolfi *et al.*, 2009).

Both of these species are green marine microalgae and the lipid content is in the range of 31-68% for *Nannochloropsis* sp. and 15-23% for *Tetraselmis* sp. (Chisti, 2007). The lipid content of microalgae is influenced by environmental conditions for example culture age, pH, temperature, salinity, nutrient limit and light intensity (Boussiba *et al.* 1987). Generally, microalgae are photoautotroph, making light an important factor for their growth (Adir *et al.*, 2003; Ragni *et al.*, 2008). Thus, light intensity (quantity) and light spectral quality (wavelength) are important factors to be considered in microalgae cultivation. Energy absorption by photoautotroph depends on the chemical property of their constitutive pigment in chlorophyll (Carvalho *et al.*, 2011). Generally, green pigments have two main absorption bands: blue or blue - green (450 - 475nm) and red (630-675nm). Therefore, the growth rate of microalgae can be improved via controlling red light or blue light (Korbee *et al.*, 2005).

When cultivating microalgae outdoors, the light intensity of sunlight varies greatly depending on the season, weather conditions, location and operating time. Thus, the light provided from direct sunlight is not constant and is unreliable for continuous and sustainable microalgae growth. Solar panels are able to utilize the sun's solar radiation; converting it into storable and utilizable electricity allowing microalgae cultivation to continue but sunlight supply is not constant. In some cases wind power is supplied to complement the solar panel system but this requires high capital cost. In indoor cultivation, fluorescent lamps are chosen as the light source for microalgae, which again requires high power consumption and higher operating cost. The replacement of these fluorescent lamps with multi LED light source will decrease the electricity consumption by 50 % (from 40.32 to 20.16 kW). Among the light source currently available, light-emitting diodes (LEDs) are the only ones that can meet the economically viable requirements. Besides, LEDs are light and small and can be fitted into virtually any photo bioreactor. Their other benefits include longer life span compared with fluorescent lamps, lower heat production, higher conversion efficiency and can tolerate on / off electric switching (Chun *et al.*, 2011).

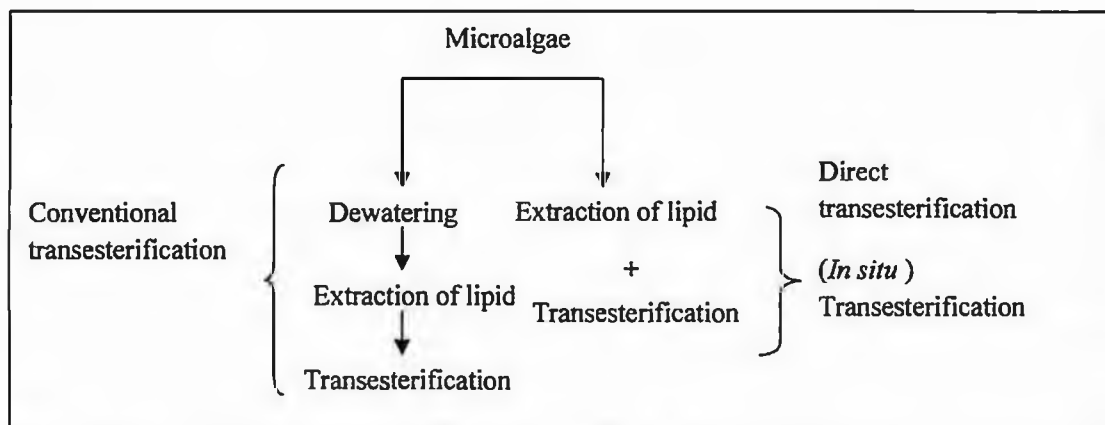
In many of literature, a variety of extraction methods were used to extract the lipids from marine microalgae and the most popular methods are oil press, supercritical fluid extraction, liquid-liquid extraction and ultrasound methods (Popoola and Yangomodou, 2006). In most of these extraction methods, the approaches were based on selective destruction of the cell wall, using abrasives, pressurized fluid extraction, microwave and enzyme (Lee *et al.*, 2010; Ranjan *et al.*, 2010). The reaction for each of these techniques is

dissimilar but the main objective is to break or decompose cell walls so as to release the crude oil contained in the cytoplasm.

Recently microwave heating (MWH) extraction has been used to extract crude oil from marine microalgae using conventional solvents (Lee *et al.*, 2010). It is a non-contact heat source, which heats the overall target reactant simultaneously as compared to conductive heating. Microwave heating was discovered for extraction of chemicals from environmental matrices (Freyburger *et al.*, 1988; Luque and Gracia, 1998; Priego and Luque, 2005; Viot *et al.*, 2007). In some previous studies of solvent extraction method, mass transfer happened from the inside to the outside, while heat transfer happened from the outside to the inside. In microwave assisted solvent extraction, mass and heat transports happened from the inside of the extracted material to the bulk solvent (Viot *et al.*, 2008). The effect of microwave irradiation is very much dependent on the intensity/frequency of the microwave irradiation. Reaction rates were enhanced (2 min instead of 2 h process reaction) upon application of radio frequency microwave energy; therefore offering a rapid and simple way to access the biomass. The field of radio frequencies range from very high frequency (VHF) (30 -300 MHz to ultra high frequency (UHF) (300 and 3000 MHz) while the term microwave is typically used for frequencies between 3 and 300 GHz (David, 2012). Unlike direct conventional heating (CH), MWH causes hot spots that lead to superheating effect. Microwave irradiation plays two roles in the synthesis process; non-thermal and thermal effects (Haswell and Howarth, 1999). Uneven microwave energy distribution and non-uniform increasing temperature were problems encountered in pulsed mode microwave irradiation, thus a continuous microwave irradiation mode was preferred (Baghurst and Mingos, 1992).

A novel technology; simultaneous cooling and microwave heating (SCMH) allows for higher levels of microwave energy to be introduced into a reaction mixture. Several researchers have reported the benefit of SCMh over microwave assisted synthesis alone and conventional heating. Maximum 85% yield of lactides (Idris *et al.*, 2012) was obtained under SCMh. In another study, it is reported that rapid synthesis of ketoamides occurred under SCMh; both aromatic and aliphatic acyl chlorides provided good to moderate yields (Chen and Deshpande, 2003). In principle, SCMh allows for higher levels of microwave energy to be introduced into a reaction and at the same time maintaining the reaction at a specific constant temperature. However the potential of SCMh technology has never been explored yet in biodiesel synthesis.

Conventionally the microalgae is first harvested via the dewatering process and then the lipids is extracted using the cell disruption methods as mentioned earlier. The lipids extracted is then converted into biodiesel via the alkali transesterification process where the biodiesel is basically extracted via three steps; namely dewatering, extraction and transesterification as depicted in Figure 1. 1



**Figure 1.1** Biodiesel production using conventional transesterification and one step transesterification

The direct transesterification (DT) process was introduced so as to reduce the number of processes, shorten the reaction time by combining the extraction and transesterification steps; where the lipid is directly extracted from the wet biomass and then transesterified into biodiesel. The direct transesterification process offers several benefits over the conventional biodiesel process where reaction steps, processing time and cost can be minimized, (Shuit *et al.*, 2010). These efforts were made by several researchers to eliminate separate extraction steps by introducing direct transesterification, also called *in situ* transesterification. The direct transesterification can be classified in a 1 step or 2 step transesterification. This technology also eliminates the dewatering process and also contributes to the reduction in the amount of extracting solvent used (Wahlen *et al.*, 2011).

Recently, Johnson and Wen (2009) revealed that the one step transesterification used on *Schizochytrium limacinum* produced higher yield of biodiesel (63.47%), consumed less time than conventional methods and the potential of lipids loss can be avoided during the extraction process. Besides, there were reported studies on the optimization of the one step transesterification investigating the influence of a variety of parameters such as the effect of catalyst concentration, amount of methanol, reaction temperature and reaction

time (Patil *et al.*, 2011; Zhang *et al.*, 2010; Jeong *et al.*, 2009) using response surface methodology. Although MWH has been used for extraction of lipids from microalgae, its use has not been extensively used in the DT of lipids from microalgae.

## 1.2 Statement of Problem

Biodiesel has become an attractive alternative energy source to replace fossil fuels but there are some significant technical challenges when cultivating microalgae indoors and also in the downstream processes. When cultivating marine microalgae which are photoautotrophic, light wavelength and intensity are critical factors which influence the biomass and neutral lipids synthesis at the cultivation stage.

Researchers (Wahidin *et al.*, 2013, Cheng and Zhang, 2013) have reported on photoperiod and intensity of white fluorescent light. Wahidin *et al.* (2013) revealed *Nannochloropsis* sp. grew favorably (cell concentration of  $6.5 \times 10^7$  cells  $\text{mL}^{-1}$ ) under light intensity of  $100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  and photoperiod of 18 h light: 6 h dark cycle. Shu *et al.* (2012) also reported that *Chlorella* sp. grew well under blue LED light intensity of 1000 lux at a temperature of 28 °C for the 24:00 h light and dark cycles producing high oil content (88 mg/L) and biomass (745 mg/L). Several studies have also reported that the optimal wavelength condition could influence the growth rate from species to species (Katsuda *et al.*, 2004). However influence of quality and quantity of LED on the growth rate and lipid content of species such as *Tetraselmis* sp. and *Nannochloropsis* sp. have not been extensively studied.

Generally conventional biodiesel production from microalgae is beleaguered with issues such as the expensive dewatering and drying process followed by the tedious extraction process utilizing large volumes of solvent. In recent years, the direct transesterification (DT) also known as *in situ* transesterification was introduced to remove dewatering and drying process so as to reduce the processing steps thus reducing the cost of biodiesel production. The DT can be categorised as the one step transesterification (OST) and the two step transesterification (TST). However the challenge lies in improving the yields and productivity of the biodiesel produced, processing time and cost of biodiesel production. Some researchers have used other methods such as supercritical (Prafulla *et al.*,



2012) which require high pressure and ultrasonic assisted extractions (Glacio *et al.*, 2013) which is time consuming.

Thus our approach is to apply microwave heating to some of the current conventional extraction of microalgae oil such as solvent extraction chloroform-methanol (Bligh and Dyer, 1959), isopropanol-hexane (Hara and Radin, 1978) and dichlorometane-methanol (Chen *et al.*, 1981) and also the DT. Besides the work of Lee *et al.* (2010) and Wahidin *et al.* (2014) the application of microwave heating (MWH) has not been extensively explored in the production of biodiesel from microalgae. In fact the use of MWH in the DT of biodiesel was not extensively explored probably because microwave energy distribution during reaction can be uneven and the increase in temperature can be unstable. Thus this study investigates the use of MWH instead of conventional heating using the current extraction methods and DT methods. Also another novel technique, simultaneous cooling microwave heating (SCMH) was introduced so as to further improve the microwave uneven energy distribution during reaction and the unstable increase in temperature.

### 1.3 Objective of Research

The aim of this study is to improve the biomass and lipid content of microalgae; namely: *Tetraselmis* sp. and *Nannochloropsis* sp. during cultivation using LED lights of various wavelengths and intensities. In addition, an attempt was also made to improve the biodiesel yields by modifying the current extraction method which was usually performed under conventional method with the microwave irradiation (MWH). Also the recent direct transesterification process was modified by applying MWH and also the simultaneous cooling microwave heating (SCMH) so as to further improve the microwave uneven energy distribution during reaction and the unstable increase in temperature. In order to achieve the objectives, the work encompasses the following:

1. To study the influence of different LED lights of various wavelengths on the amount of biomass and lipid content; and the results were compared with white fluorescent light as the standard.

2. To investigate the relationship between the quality and intensity of LED illumination with the fatty acid methyl esters (FAME's) produced.
3. To investigate the influence of microwave heating on the current conventional extraction of microalgae oil such as solvent extraction isopropanol-hexane (Hara and Radin, 1978), chloroform-methanol (Bligh and Dyer, 1959), chloroform-methanol (Folch *et al.*, 1957) and dichlorometane-methanol (Chen *et al.*, 1981) and compare the results with the control (conventional heating); all performed in a water bath. The comparison is based on the biodiesel yields and quality of biodiesel.
4. To investigate the influence of microwave heating on the direct transesterification for both the OST and TST in terms of quantity and quality of FAMEs. The influence of the various combined catalyst was also studied.
5. To investigate the influence of simultaneous cooling and microwave heating (SCMH) method on the most common transesterification method and DT taking into consideration, parameters such as reaction temperature, microwave energy input, water content, duration of transesterification reaction, lipids to methanol ratio and cooling effect.

#### 1.4 Scope of Research

The research was conducted within the following limits:

- i) The different LED wavelengths used are blue (457nm), red (660nm) and red mix blue (50%:50%) and the intensities used are 100, 150 and 200  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ .
- ii) The current extraction methods used and modified are Hara and Radin (1978), Folch *et al.* (1957), Chen *et al.* (1981) and Bligh and Dyer (1959) methods. The conventional heating method was performed at a temperature of 100°C and duration of around 30-60 minutes with stirring speed of 400rpm. The microwave heating was performed at 500 W, temperature 65°C and duration 10 minutes.

- iii) The transesterification of biodiesel through SCM<sub>H</sub> was performed in a microwave reactor which was cooled using water jacket. Several factors such as temperature (30 - 70°C), power (500 - 900W) and reaction duration (8h - 30h), methanol ratio (1:4, 1:6, 1:8, 1:10 and 1:12), water effect (0 - 16ml) and cooling effect (4 - 35°C) were investigated.
- iv) The direct transesterification (OST and TST) of biodiesel through microwave heating method was performed in a microwave reactor and different types of combined catalysts (NaOH, HCl and H<sub>2</sub>SO<sub>4</sub>) were used.
- v) Simultaneous cooling and microwave heating with one step biodiesel production was studied.
- vi) The biomass and biodiesel yields were determined and quality of FAMEs from marine microalgae was analyzed using Gas Chromatography.

The schematic flow chart of experiment approach was depicted in Figure 1.2.

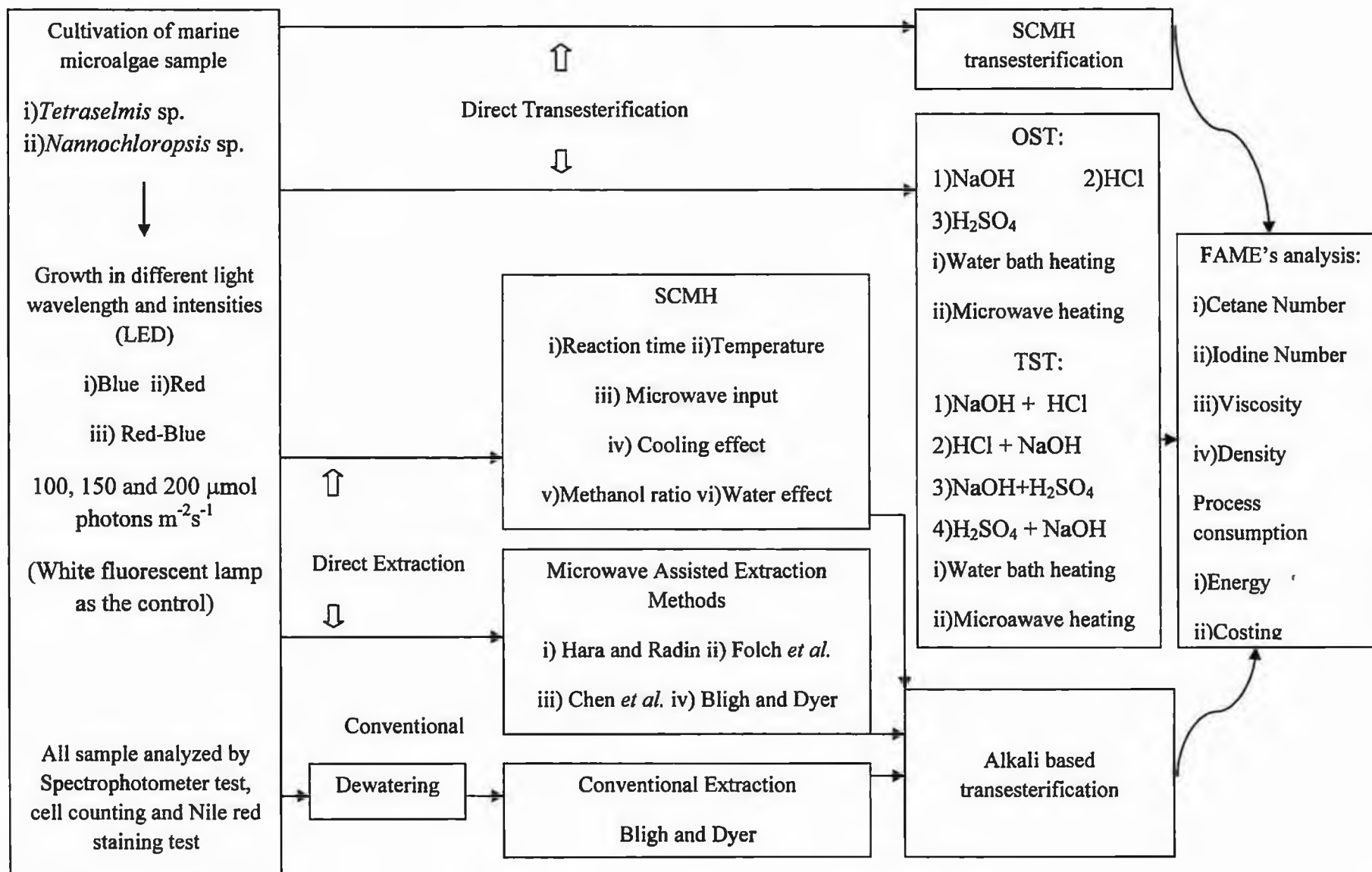


Figure 1.2 Schematic flow chart of experiment approach.

## 1.5 Research Significance

The novelty of this study lies in the judicious use of a tri-combination of process improvement using LED lighting system to overcome unstable light source from sunlight for cultivating *Tetraselmis* sp. and *Nannochloropsis* sp. outdoor. Secondly, the MWH was introduced to the current extraction method and also the DT method so as to increase biodiesel's quality and quantity at reduced energy consumption. Finally the novel SCMH transesterification was also used in the DT process also to further improve the biodiesel yield and productivity. The findings achieved in this study will provide important information on biodiesel production via MWH and SCMH in the DT process of microalgae.

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