NEW MICROWAVE BASED TRANSESTERIFICATION TECHNIQUES FOR BIODIESEL PRODUCTION FROM CULTIVATED MICROALGAE

TEO CHEE LOONG

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Faculty of Chemical Engineering
Universiti Teknologi Malaysia

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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 μmol photons $m^{-2}s^{-1}$. 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 μmol photons $m^{-2}s^{-1}$) 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_2SO_4) 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 SCMH 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 SCMH at optimum setting, the biodiesel yield was more than 3.75 folds than conventional method. Gas chromatography analysis depicted that the biodiesel produced from SCMW 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 SCMW 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 μmol fatons $m^{-2}s^{-1}$ 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 μmol foton $m^{-2}s^{-1}$) menghasilkan biojisim dan lipid tertinggi. Beberapa teknik telah digunakan untuk memperbaiki kaedah DT i) teknik pemanasan gelombang mikro (MWH) kepada pelbagai kaedah pengekstrakan, ii) penggunan 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_2SO_4) 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_3H_7OH	-	Iso-proponol
C_6H_{14}	-	Hexane
$CHCl_3$	-	Chloroform
CH_3OH	-	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_2SO_4	-	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
$NaH_2PO_4.2H_2O$ - Dihydrate sodium dihydrogen phosphate
$NaNO_3$ - 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 *in situ* 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^{-1} - Cells per milliliter
DW - Dry weight
GHz - Giga hertz
g/L/ day - Gram per litre per day
g / m^2/ 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^2/ day - Gram dry weight per square meter per day
g DW/ m^2/ h - Gram dry weight per square meter per hectare
H - Hour
kJ - Kilo joule
kW - Kilo watt
L - Litre
L m^{-2}d^{-1} - 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^{-1} year^{-1} - Tonne per hectare per year

TWh - TeraWatt-hour

μ mol photon m^{-2} s^{-1} - Micro mol photon per mili square per second

v/v - Volume per volume

W - Watt

W/ m^{2} - 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; Virot *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 (Virot *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

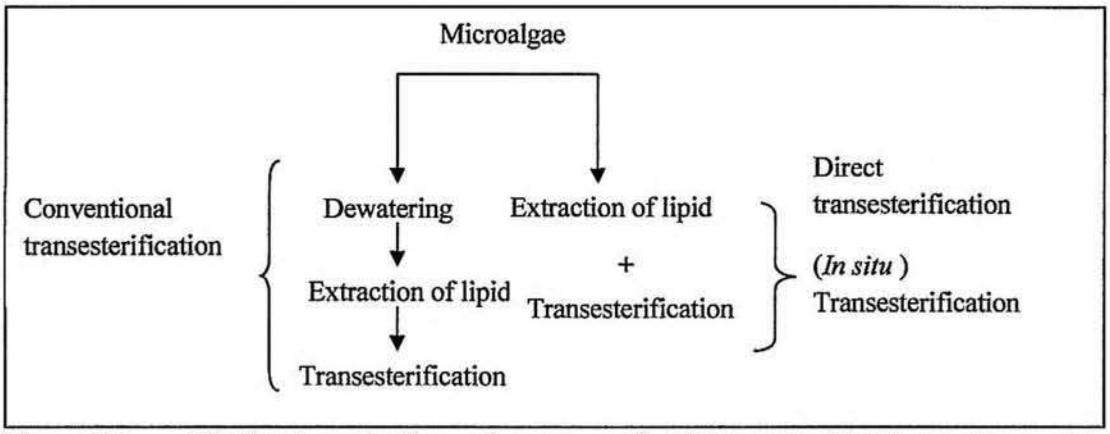

Figure 1.1 Biodiesel production using conventional transesterification and one step transesterification

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 6.5×10^7 cells ml^{-1}) under light intensity of 100 μmol photons m^{-2} 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 (Kastsuda *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 μmol photons $m^{-2}s^{-1}$.

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 SCMH 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_2SO_4) 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.

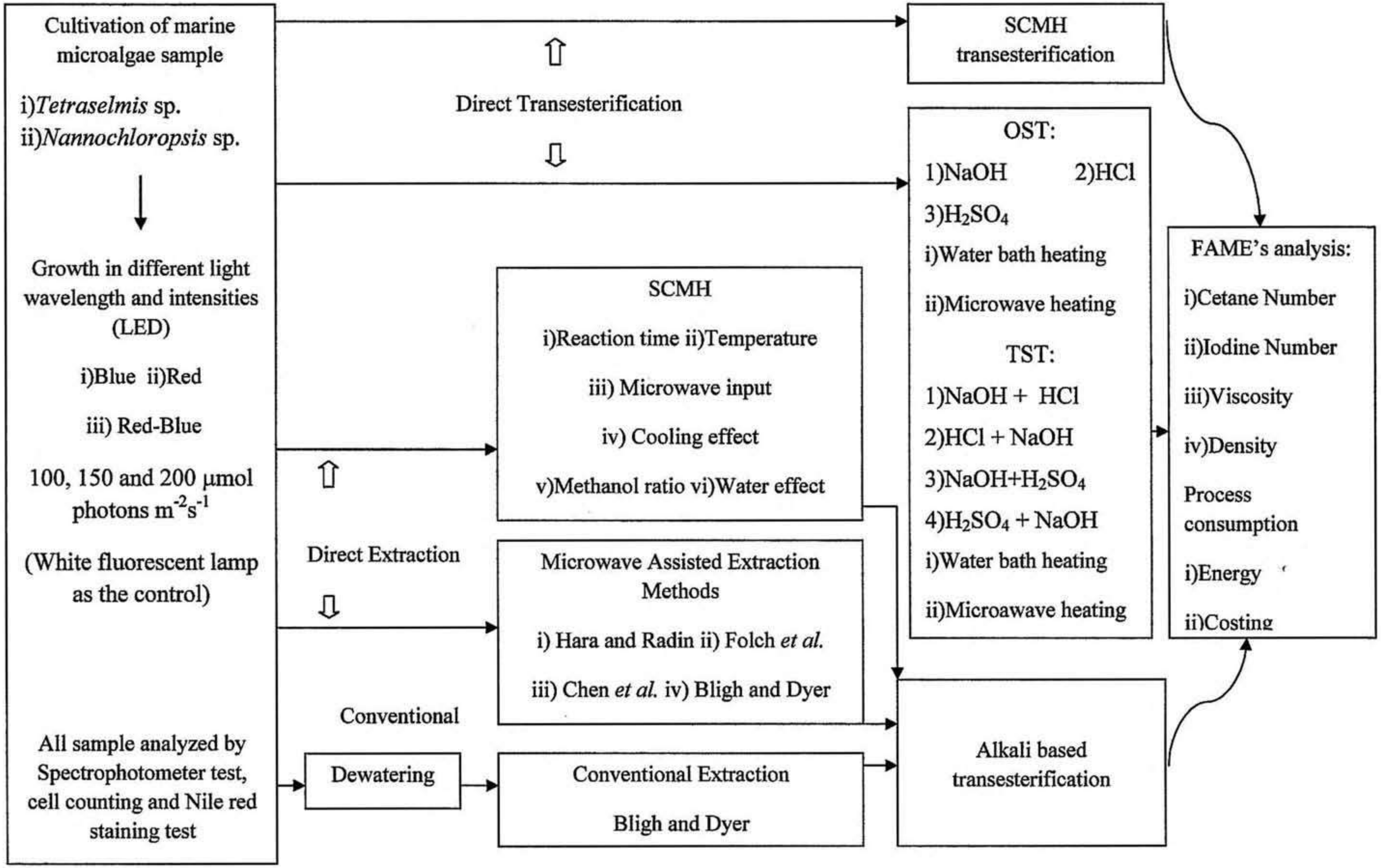

Figure 1.2 Schematic flow chart of experiment approach.

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.

REFERENCES

Aage, S., Laetitia, B., Ane, B., Nils, B., Michele, H. G., Guro, H., Eivind, H., Marius, H., Christine, K., Hanne, B. L., Håvard, L., Konrad, P., Elisabeth, S., and Bjørn, U. (2008). How to Combat Global Warming - An ambitious but necessary approach to reduce greenhouse gas emissions, *Bellona*, June 2008 Norway, 51.

Adir, N., Zer, H., Shochat, S. and Ohad, I. (2003). Photoinhibition – a historical perspective. *Photosynthesis Research*. 76, 343–370.

Akiya, N. and Savage, P.E. (2002). Roles of water for chemical reactions in high-temperature water. *Chemical Reviews*. 102(8), 2725–2750.

Allakhverdiev, I.S., Sakamoto, A., Nishiyama, Y., Inaba, M. and Murata, N. (2000). Ionic and osmotic effects of NaCl-induced inactivation of photosystems I and II in *Synechococcus* sp. *Plant Physiology*. 123 (3), 1047–1056.

Amaro, H.M., Guedes, C.A. and Malcata, X.F. (2011). Advances and perspectives in using microalgae to produce biodiesel. *Applied Energy*. 88, 3402–3410.

Andrich, G., Zinnai, A., Nesti, U., Venturi, F. and Fiorentini, R. (2006). Supercritical fluid extraction of oil from microalga *Spirulina (Arthrospira) platensis*. *Acta Alimentaria*. 35, 195–203.

Aresta, M., Dibenedetto, A., Carone, M., Colonna, T. and Fragale, C. (2005). Production of biodiesel from macroalgae by supercritical CO_2 extraction and thermochemical liquefaction. *Environment Chemistry Letters*. 3, 136–139.

Arvela, R. K. and Leadbeater, N. E. (2005). Suzuki coupling of aryl chlorides with phenylboronic acid in water, using microwave heating with simultaneous cooling. *Organic Letters*. 7, 2101-2104.

Asulabh, K.S., Supriya, G., Ramachandra, T.V. (2012). Effect of salinity concentrations on growth rate and lipid concentration in *Microcystis* sp., *Chlorococcum* sp. and *Chaetoceros* sp. In: LAKE: *National Conference on Conservation and Management of Wetland Ecosystems*. 27–32.

Atta, M., Idris, A., Bukhari, A. and Wahidin, S. (2013). Intensity of blue LED light: A potential stimulus for biomass and lipid content in fresh water microalgae *Chlorella vulgaris*. *Bioresource Technology*. 148, 373–378.

Azachi, M., Sadka, A., Fisher, M., Goldshlag, P., Gokhman, I. and Zamir, A. (2002). Salt induction of fatty acid elongase and membrane lipid modifications in the extreme halotolerant alga *Dunaliella salina*. *Plant Physiology*. 129, 1320–1329.

Azcan, N. and Danisman, A. (2007). Alkali catalyzed transesterification of cottonseed oil by microwave irradiation. *Fuel*. 86, 2639–2644.

Baba, M. and Shiraiwa, Y. (2012). High-CO_2 response mechanisms in microalgae. In: Najafpour, M.M. (Ed.), *Advances in Photosynthesis-Fundamental Aspects*. (pp. 12–435). New York: In Tech.

Babcock, R.W., Malda, J. and Radway, J.C. (2002). Hydrodynamics and mass transfer in a tubular airlift photobioreactor. *Journal Applied Phycology*. 14, 169–184.

Baghurst, D. R. and Mingos, D. M. P. (1992). Superheating effects associated with microwave dielectric heating. *Journal Chemical Society Chemical Communications*. 674-677.

Barbosa, M.J., Albrecht, M., Wijffels, R.H. (2003). Hydrodynamic stress and lethal events in sparged microalgae cultures. *Biotechnology Bioengineering*. 83, 112–120.

Barnabas, I.J., Dean, J.R., Fowlis, I.A. and Owen, S.P. (1995). Extraction of polycyclic hydrocarbons from highly contaminated soils using microwave energy. *Analyst*. 120, 1897–1904.

Becker, E. W. (1994). *Microalgae. Biotechnology and Microbiology*. United Kingdom: Cambridge University Press

Berges, J., Franklin, D. and Harrison, P. (2001). Evolution of an artificial seawater medium: improvements in enriched seawater, artificial water over the last two decades, *Journal of Phycology*. 37, 1138–1145.

Bergman, J.W., Carlson, G., Kushnak, G., Riveland, N.R., Stallknecht, G., Welty, L.E. and Wichman, D. (1989). Registration of Finch Safflower. *Crop Science*. 29, 829–832.

Berrios, M, Gutiérrez, M.C., Martín, M.A. and Martín, A. (2009). Application of the factorial design of experiments to biodiesel production from lard. *Fuel Process Technology*. 90, 1447–1451.

Bligh, E.G. and Dyer, W.J. (1959). A rapid method of total lipid extraction and purification, *Canadian Journal Biochemistry Physiology*. 37, 911–917.

Boussiba, S.,Vonshak, A., Cohen, Z., Avissar, Y. and Richmond, A. (1987). Lipid and biomass production by the halotolerent microalga *Nanochloropsis salina*. *Biomass*. 12 (1), 37-47.

Brennan, L. and Owende, P. (2010). Biofuels from microalgae: a review of technologies for production, processing, and extractions of biofuels and co-products. *Renewable and Sustainable Energy Reviews*. 14, 557–577.

Canela, A.P.R.F., Rosa, P.T.V., Marques, M.O.M. and Meireles, M.A.A. (2002). Supercritical fluid extraction of fatty acids and carotenoids from the microalgae *Spirulina maxima*. *Industry Engineering Chemisty Research*. 41, 3012–3018.

Carballo, C.E.C., Tuan, P.M., Jansen, M. and Wijfells, R.H. (2003). Vitamin E (tocopherol) production by the marine microalgae *Dunaliella tertiolecta* and *Tetraselmis suecica* in batch cultivation. *Biomolecular Engineering*. 20, 139–147.

Carlozzi, P. (2003). Dilution of solar radiation through "culture" lamination in photobioreactor rows facing south–north: a way to improve the efficiency of light utilization by cyanobacteria *(Arthrospira platensis)*. *Biotechnology Bioengineering*. 81(3), 305–315.

Carvalho, A.P., Silva, S.O., Baptista, J.M., and Malcata, F.X. (2011). Light requirements in microalgal photobioreactors: an overview of biophotonic aspects. *Applied Microbiology and Biotechnology*. 89(5), 1275–1288.

Chaloub, R.M., Silva, L.M.T., Rodrigues, M.A. and dos Santos, C.P. (2003). Phase transition of thylakoid membranes modulates photoinhibition in the cyanobacterium *Anabaena siamensis*. *Photosynthesis Research*. 78, 143-152.

Cheirsilp, B. and Torpee, S. (2012). Enhanced growth and lipid production of microalgae under mixotrophic culture condition: effect of light intensity, glucose concentration and fed-batch cultivation. *Bioresource Technology*. 110, 510–516.

Chen, C.Y., Yeh, K.Y., Aisyah, R., Lee, D.J. and Chang, J.S., (2011). Cultivation, Photobioreactor Design and Harvesting of Microalgae for Biodiesel Production: A Critical Review. *Bioresource Technology*. 102, 71–81.

Chen, I.S., Shen, C.S.J., Sheppard, A.J. (1981). Comparasion of methylene chloride and chloroform for the extraction of fats from food products. *Journal of American Oil Chemists's Society*. 58, 599–601.

Chen, J.J. and Deshpande, S.V. (2003). Rapid synthesis of ketoamides using microwave irradiation- simultaneous cooling method. *Tetrahedron Letters*. 44, 8873-8876.

Chen, W., Sommerfeld, M., Hu, Q. (2011). Microwave-assisted Nile red method for in vivo quantification of neutral lipids in microalgae. *Bioresource Technology*. 102, 135–141.

Chen, Y., Xiao, B., Chang, J., Fu, Y., Lv, P. and Wang, X. (2009). Synthesis of biodiesel from waste cooking oil using immobilized lipase in fix bed reactor. *Energy Conversation Management*. 50, 668–673.

Cheng, J., Sun, J., Huang, J., Feng, J., Zhou, J.H. and Kefa, C. (2013). Dynamic microstructures and fractal characterization of cell wall disruption for microwave irradiation-assisted lipid extraction from wet microalgae. *Bioresource Technology*. 150, 67–72.

Cheng, Y. and Zheng, Z. (2013). Performance of photoperiod and light intensity on biogas upgrade and biogas effluent nutrient reduction by the microalgae *Chlorella* sp. *Bioresource Technology*. 139, 292–299.

Chisti, Y. (2007). Biodiesel from microalgae. *Biotechnology Advanced*. 25, 294-306.

Chisti, Y. (2008). Biodiesel from microalgae beats bioethanol. *Trends Biotechnology*. 26, 126-131.

Choi, I., Choi, S.J., Chun, J.K. and Moon, T.W. (2006). Extraction yield of soluble protein and microstructure of soybean affected by microwave heating. *Food Process Preservation*. 30, 407–419.

Chun, Y. C., Kuei, L. Y., Rifka, A., Duu, J. L. and Jo, S. C. (2011). Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: A critical review. *Bioresource Technology*. 102, 71–81.

Choi, S.L., Suh, I.S. and Lee, C.G. (2003). Lumostatic operation of bubble column photobioreactors for Haematococcus pluvialis cultures using a specific light uptake rate as a control parameter. *Enzyme and Microbial Technology*. 33, 403–409.

Christenson, L. and Sims, R. (2011). Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. *Biotechnology Advance*. 29, 686–702.

Converti, A., Casazza, A.A., Ortiz, E.Y., Perego, P. and Del Borghi, M. (2009). Effect of temperature and nitrogen concentration on the growth and lipid content of *Nannochloropsis oculata* and *Chlorella vulgaris* for biodiesel production. *Chemical Engineering Processing*. 48, 1146–1151.

Cossins, A.R. (1994). Homeoviscous adaptation of biological membranes and its functional significance. In: Cossins AR, editor. *Temperature Adaptation of Biological Membranes* (pp 63-76). London: Portland Press.

Da Silva, R.B., Lima Neto, A.F., Soares dos Santos, L.S., de Oliveira Lima, J.R., Chaves, M.H. and dos Santos, Jr. J.R (2008). Catalysts of Cu(II) and Co(II) ions adsorbed in chitosan used in transesterification of soy bean and babassu oils – A new route for biodiesel syntheses. *Bioresource Technology*. 99, 6793–6798.

Danquah, M.K., Gladman, B., Moheimani,N. and Forde, G.M. (2009). Microalgal growth characteristics and subsequent influence on dewatering efficiency. *Chemical Engineering Journal*. 151, 73–78.

Das, P., Lei, W., Aziz, S.S. and Obbard, J.P. (2011). Enhanced algae growth in both phototrophic and mixotrophic culture under blue light. *Bioresource Technology*. 102, 3883–3887.

David, C., Matteo, P., David, C., Mario, R. T., Liliana, R., Niccolò, B., Graziella, C. Z. and Paolo, B. (2013). Review of energy balance in raceway ponds for microalgae cultivation: Re-thinking a traditional system is possible. *Applied Energy*. 102, 101–111.

David, M. P. (2012). *Microwave Engineering* (Fourth Edition). United State: John Wiley & Sons, Inc.

Day, J., Edwards, A. and Rodgers, G. (1991). Development of an industrial-scale process for the heterotrophic production of a micro-algal mollusc feed. *Bioresource Technology*. 38, 245–249.

de Boer, M.K., Koolmees, E.M., Vrieling, E.G., Breeman, A.M. and van Rijssel, M. (2005). Temperature responses of three Fibrocaps japonica strains (*Raphidophyceae*) from different climate regions. *Journal of Plankton Research*. 27, 47–60.

Degen, J., Uebele, A., Retze, A., Schmidt, S. U. and Trosch, W. (2001). A novel airlift photobioreactor with baffles for improved light utilization through the flashing light effect. *Journal Biotechnology*. 92, 89–94.

Demirbas, A. (2003). Biodiesel fuels from vegetable oils via catalytic and noncatalytic supercritical alcohol transesterifications and other methods: a survey. *Energy Conversation Management*. 44, 2093–2109.

Demirbas, A.(2009). Production of biodiesel from algae oils. Energy Sources Part A-Recovery Utilization. *Environment Effect*. 31, 163–168.

Deng, X., Li, Y. and Fei, X. (2009). Microalgae: a promising feedstock for biodiesel. *African Journal Microbiology Res*earch. 3(13), 1008–1014.

Di Serio, M., Cozzolino, M., Tesser, R., Patrono, P., Pinzari, F. and Bonelli, B. (2007). Vanadyl phosphate catalysts in biodiesel production.. *Applied Catalysis A: General*. 320, 1–7.

Dintzner, M.R., Lyons, T.W., Akroush, M.K. and Wucka, P., *Rzepka*, A.T. (2005). Quick, Clean and Green Synthesis of Methylenedioxy precocene and other Chromenes over Basic Montmorillonite K10 Clay. *Synlett* 5, 785–788.

Dismukes, G.C., Carrieri, D., Bennette, N., Ananyev, G.M. and Posewitz, M.C. (2008). Aquatic phototrophs: efficient alternatives to land-based crops for biofuels. *Current Opinion Biotechnology*. 19(3), 235–240.

Dizge, N. and Keskinler, B. (2008). Enzymatic production of biodiesel from canola oil using immobilized lipase. *Biomass Bioenergy*. 32, 1274–1278.

Dizge, N., Aydiner, C., Imer, D.Y., Bayramoglu, M., Tanriseven, A. and Keskinler, B. (2009). Biodiesel production from sunflower, soybean, and waste cooking oils by transesterification using lipase immobilized onto a novel microporous polymer. *Bioresource Technology*. 100, 1983–1991.

Doucha, J., and Lvansk, K. (2008). Outdoor open thin-layer microalgal photobioreactor: potential productivity. *Journal of Applied Phycology*, 21(1), 111-117.

Edith, M. G., Veera, G. G., Andro, M., William, H. and Rafael, H. (2014). Extractive-transesterification of algal lipids under microwave irradiation with hexane as solvent. *Bioresource Technology*. 156, 240–247.

Fajardo, A.R., Cerdan, L.E., Medina, A.R., Fernandez, F.G.A., Moreno, P.A.G. and Grima, E.M. (2007). Lipid extraction from the microalga *Phaeodactylum tricornutum*. *Europe Journal Lipid Science Technology*. 109, 120–126.

Feng, P.Z., Deng, Z.Y., Fan, L. and Hu, Z.Y. (2012). Lipid accumulation and growth characteristics of Chlorella zofingiensis under different nitrate and phosphate concentrations. *Journal Bioscience Bioengineering*. 102, 106–110.

Fleischer, W.E. (1935). The relation between chlorophyll content and rate of photosynthesis. *The Journal of General Physiology*. 18, 573–597.

Folch, J., Less, M. and Stanley, G.H.S. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal Biology Chemistry*. 226, 497– 509.

Freyburger, G., Heape, A., Gin, H., Boisseau, M. and Cassagne, C. (1988). Decrease of lipid extractability by chloroform–methanol upon addition of water to human erythrocytes. *Analytical Biochemistry*. 171, 213–216.

Friedrich, M.S. (2004, August 2). A worlwide review of the commercial production of biodiesel – a technological, economic and ecological investigation based on case studies. *Department of Technology and Sustainable Product Management (ITNP), Wien*, Retrieved August 6, 2004, from <itnp.wu-wien.ac.at/archiv_doc/BAND41_ FRIEDRICH.PDF>.

Fukuda, H., Kondo, A. and Noda, H. (2001). Biodiesel fuel production by transesterification of oils. *Journal Bioscience Bioengineering*. 2(5), 405–416.

Fu, Y. F., Wen, Y., Gui, Z. J., Yi, N. X. and Ting, Y. K. (2005). Enhancement of fatty acid production of *Chlorella* sp. (Chlorophyceae) by addition of glucose and sodium thiosulphate to culture medium. *Process Biochemistry*. 40, 1315–1318.

George, B., Pancha, I., Desai, C., Chokshi, K., Paliwal, C., Ghosh, T. and Mishra, S. (2014). Effects of different media composition, light intensity and photoperiod on morphology and physiology of freshwater microalgae *Ankistrodesmus falcatus* – a potential strain for bio-fuel production. *Bioresource Technology*. 171, 367–374.

Glacio, S. A., Leonardo, J.B.L. Matos, J. O., Fernandes, Samuel. J.M.C., Luciana, R.B.G., Fabiano, A.N.F. and Wladimir, R.L.F. (2013). Extraction of lipids from microalgae by ultrasound application: Prospection of the optimal extraction method. *Ultrasonics Sonochemistry*. 20, 95–98.

Greenwell, H.C., Laurens, L.M.L., Shields, R.J., Lovitt, R.W. and Flynn, K.J. (2010). Placing microalgae on the biofuels priority list: a review of the technological challenges. *Journal of Royal Society Interface*. 7, 703–726.

Griffiths, M.J. and Harrison, S.T.L. (2009). Lipid productivity as a key characteristic for choosing algal species for biodiesel production. *Journal of Applied Phycology*. 21, 493–507.

Griffiths, M.J., Hillevan, R.P. and Harrison, S.T.L. (2010). Selection of direct transesterification as the preferred method for assay of fatty acid content of microalgae. *Lipids*. 45, 1053-1060.

Griffiths, M.J., Dicks, R., Richardson, C. and Harrison, S. (2011). Advantages and challenges of microalgae as a source of oil for biodiesel. In: Stoytcheva, M., Montero, J., editors. *Biodiesel– Feed Stocks and Processing Technologies* (pp. 177–92). Rijeka: In Tech.

Grobbelaar, J.U. and Kurano, N. (2003). Use of photoacclimation in the design of a novel photobioreactor to achieve high yields in algal mass cultivation. *Journal Applied Phycology*. 15, 121–126.

Grossman, A.R., Croft, M., Gladyshev, V.N., Merchant, S.S., Posewitz, M.C, Prochnik S. and Spalding, M.H. (2007). Novel metabolism in *Chlamydomonas* through the lens of genomics. *Current Opinion in Plant Biology*. 10, 190–198.

Gouveia, L. and Oliveira, A.C. (2009). Microalgae as a raw material for biofuels production. *Jorunal Indian Microbiology Biotechnology*. 36, 269–274.

Gudin, C. and Chaumont, D. (1991). Cell fragility: The key problem of microalgae mass production in closed photobioreactors. *Bioresource Technology*. 38, 145-151.

Guillard, R.R.L, Ryther J.H.. (1962) Studies of marine planktonic diatoms. *I. Cyclotella nana Hustedt* and *Detonula confervacea* (Cleve). *Canadian Journal of Microbiology*. 8, 229-239.

Hall, D.O., Fernandez, F.G.A., Guerrero, E.C., Rao, K.K. and Grima, E.M. (2003). Outdoor helical tubular photobioreactors for microalgal production: modeling of fluid-dynamics and mass transfer and assessment of biomass productivity. *Biotechnology Bioengineering*. 82(1), 62–73.

Hallenbeck, P.C. and Benemann, J.R. (2002). Biological hydrogen production: fundamentals and limiting processes. *Journal Hydrogen Energy.* 27, 1185–1193.

Halim, R., Gladman, B., Danquah, M.K. and Webley, P.A. (2011). Oil extraction from microalgae for biodiesel production. *Bioresoure Technology.* 102, 178–185.

Hara, A. and Radin, N.S. (1978). Lipid extraction of tissues with a low-toxicity solvent. *Analytical Biochemistry.* 90, 420–423.

Harino, H., Fukushima, M. and Kawai, S. (2000). Accumulation of butyltin and phenyltin compounds in various fish species. *Archives of Environmental Contamination and Toxicology.* 39(1), 13-19.

Harwood, J.L. (2004). Membrane Lipids in Algae. *In Lipids in Photosynthesis: Structure, Function and Genetics* (pp. 53–64). Netherlands: Springer.

Haswell, S. J. and Howarth, N (1999). Development of a bi-functional silica monolith for electro- osmotic pumping and DNA. *Analytical Chimica Acta.* 387, 113-120.

Hemwimon, S., Pavasant, P. and Shotipruk, A., (2007) Microwave-assisted extraction of antioxidative anthraquinones from roots of Morinda citrifolia. *Seperation and Purification Technology.* 54, 44–50.

Hibberd, D.J. (1980). Prymnesinphytes (Haptophytes). In: Phytoflagellates. E.R.Cox, Ed. (pp 273-317). New York: Elsevier North Holland Inc.

Ho, S.H., Chen, C.N.N., Lai, Y.Y., Lu, W.B. and Chang, J.S. (2014). Exploring the high lipid production potential of a thermotolerant microalga using statistical optimization and semi-continuous cultivation. *Bioresource Technology.* 163, 128–135.

Hsiao, M.C, Lin, C.C., Chang, Y.H. and Chen, L.C. (2010). Ultrasonic mixing and closed microwave irradiation assisted transesterification of soybean oil. *Fuel.* 89, 3618–3622.

Hsieh, C. H. and Wu, W. T. (2009) A novel photobioreactor with transparent rectangular chambers for cultivation of microalgae. *Biochemistry Engineering Journal.* 46, 300–305.

Hu, Q., Richmond, A. (1996). Productivity and photosynthetic efficiency of *Spirulina platensis as* affected by light intensity, algal density and rate of mixing in a flat plate photobioreactor. *Journal of Applied Phycology.* 8, 139–145.

Hu, Q., Sommerfeld, M., Jarvis, E., Ghirardi, M., Posewitz, M., Seibert, M. and Darzins, A. (2008). Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. *Plant Journal.* 54, 621–639.

Huerlimann, R., de Nys, R. and Heimann, K. (2010). Growth, lipid content, and fatty acid composition of tropical microalgae for scale-up production. *Biotechnology Bioengeenring.* 107, 245–257.

Idris, A., Attaullah, B., Noordin, M. Y. and Tan K.G. (2012). A Simultaneous cooling and dielectric heating: An advanced technology to improve the yield of lactides. *Progress in electromagnetic research symposium proceedings:* 141 -145.

Imahara, H., Minami, E. and Saka, S. (2006). Thermodynamic study of cloud point of biodiesel with its fatty acid composition. *Fuel.* 85(12–13), 1666–1670.

Ioannis, S., Efthalia, C., Spyros, F. and Theocharis, T. (2014) Optimization of biodiesel production from waste lard by a two-step transesterification process under mild conditions *Energy for Sustainable Development.* 23, 110-114

Irianto, A. and Austin, B. (2002) Probiotics in aquaculture. *Journal Fish Diseases.* 25, 633–642.

Iverson, T.M. (2006). Evolution and unique bioenergetic mechanisms in oxygenic photosynthesis. *Current Opinion Chemical Biology.* 10, 91–100.

Jacob, J. and Chia, L. H. L. (1995). Thermal and non-thermal interaction of microwave radiation with materials. *Journal Material Science.* 30, 5321-5327.

Jasvinder, S. and Sai, G. (2010). Commercialization potential of microalgae for biofuels production. *Renewable and Sustainable Energy Reviews.* 14, 2596–2610.

Javed, I. (2012). Development of cost-effective and begins lipid extraction system for microalgae, Faculty of the Louisiana State University and Agricultural and Mechanical College.

Jeong, G.T., Yang, H.S. and Park, D.H. (2009). Optimization of transesterification of animal fat ester using response surface methodology. *Bioresource Technology.* 100, 25 -30.

Jiao, L., Yanan, L., Haitao, W. and Song, X. (2015). Direct transesterification of fresh microalgal cells. *Bioresource Technology.* 176, 284–287.

Johnson, M.B. and Wen, Z.Y. (2009). Production of biodiesel fuel from the microalga *Schizochytrium limacinum* by direct transesterification of algal biomass. *Energy Fuels.* 23, 5179–5183.

Jorge, A.V.C. and Michele, G.d.M. (2014). An Open Pond System for Microalgal Cultivation. *Biofuels from Algae* (pp. 4). Elsevier B.V.: England.

Kalita, N., Baruah, G., Goswami, R.C.D., Talukdar, J. and Kalita, M.C. (2011). *Ankistrodesmus falcatus*: a promising candidate for lipid production, its biochemical analysis and strategies to enhance lipid productivity. *Journal Microbiology Biotechnology Research.* 1, 148–157.

Kalpesh, K.S., Holger, S. and Peer, M.S. (2012). High lipid induction in microalgae for biodiesel production. *Energies.* 5, 1532–1553.

Kan, G., Shi, C., Wang, X., Xie, Q., Wang, M., Wang, X. and Miao, J. (2012). Acclimatory responses to high-salt stress in *Chlamydomonas (Chlorophyta, Chlorophyceae)* from Antarctica. *Acta Oceanological Sinica.*31, 116–124.

Kang, C. D., Han, S. J., Choi, S. P. and Sim, S. J. (2010). Fed-batch culture of astaxanthin-rich *Haematococcus pluvialis* by exponential nutrient feeding and stepwise light supplementation. *Bioprocess and Biosystems Engineering*. 33, 133–139.

Kanitkar, A.V. (2010). Parameterization of Microwave Assisted Oil Extraction and its Transesterification to Biodiesel. *Master's Thesis. Lousiana State University*, Baton Rougue, LA.

Kappe, C.O. (2004). Controlled microwave heating in modern organic synthesis. *Angewandte Chemie Internation Edition*. 43, 6250–6284.

Katsuda, T., Lababpour, A., Shimahara, K. and Katoh, S. (2004). Astaxanthin production by *Haematococcus pluvialis* under illumination with LEDs. *Enzyme Microbiology Technology*. 35, 81–86.

Kawashima, A., Matsubara, K. and Honda, K. (2009). Acceleration of catalytic activity of calcium oxide for biodiesel production. *Bioresource Technology*. 100, 696–700.

Khotimchenko, S.V. and Yakovleva, I.M. (2005). Lipid composition of the red alga *Tichocarpus crinitus* exposed to different levels of photon irradiance. *Phytochemistry*. 66, 73–79.

Kim, T.H., Lee, Y., Han, S.H., Hwang, S.J. (2013). The effects of wavelength and wavelength mixing ratios on microalgae growth and nitrogen, phosphorus removal using Scenedesmus sp. for wastewater treatment. *Bioresource Technology*. 130, 75–80.

Kindle, K.L. (1990) High-frequency nuclear transformation of *Chlamydomonas reinhardtii*. *Proceeding of National Academic Science*. 87, 1228–1232. U.S.A.

Kiss, G.A.C., Forgacs, E., Cserhati, T., Mota, T., Morais, H. and Ramos, A. (2000). Optimization of the microwave-assisted extraction of pigments from *paprika (Capsicum annuum L.)* powders. *Journal Chromatography A*. 889, 41–49.

Knothe, G. (2002). Structure indices in FA chemistry. How relevant is the iodine value? *Journal of the American Oil Chemist's Society*. 9, 847–853.

Knothe, G., Dunn, R.O. and Bagby, M.O. (1997). Biodiesel: the use of vegetable oils and their derivatives as alternative Diesel fuels. *American Chemistry Society Symposium Series*. 666, 172–208.

Korbee, N., Figueroa, F. and Aguilera, J. (2005). Effect of light quality on the accumulation of photosynthetic pigments, proteins and mycosporine-like amino acids in the red alga *Porphyra leucosticte* (Bangiales, Rhodophyta). *Journal of Photochemistry and Photobiology B*. 80, 71–78.

Koberg, M., Abu, M.R. and Gedanken, A. (2011). Optimization of bio-diesel production from soybean and wastes of cooked oil: combining dielectric microwave irradiation and a SrO catalyst. *Bioresource Technology*. 102, 1073–1078.

Kondo, T., Yamamoto, H., Tatarazako, N., Kawabe, K., Koshio, M., Hirai, N. and Morita, M. (2005). Bioconcentration factor of relatively low concentrations of chlorophenols in Japanese medaka. *Chemosphere*. 61(9), 1299-1304.

Kusdiana, D. and Saka, S. (2004). Two step preparation for catalyst –free biodiesel fuel production. *Applied biochemistry biotechnology*. 115, 781-791.

Kulkarni, M.G., Gopinath, R., Meher, L.C. and Dalai, A.K. (2006). Solid acid catalyzed biodiesel production by simultaneous esterification and transesterification. *Green Chemistry*. 8, 1056–1062.

Lardon, L., Helias, A., Sialve, B., Steyer, J. and Bernard, O. (2009). Life-cycle assessment of biodiesel production from microalgae. *Environmental Science & Technology*. 43, 6475–6481.

Lauf, R.J., Bible, D.W., Johnson, A.C. and Everliegh, C.A. (1993). 2–18 GHz broadband microwave heating systems. *Microwave Journal*. 36(11), 24–27.

Lee, C.G. and Palsson, B.O. (1994). High-density algal photobioreactors using light emitting diodes. *Biotechnology Bioengineering*. 44, 1161–1167.

Lee, H. S., Kim, Z. H., Jung, S. E., Kim, J. D. and Lee, C. G. (2006). Specific light uptake rate can be served as a scale-up parameter in photobioreactor operations. *Journal of Microbiology and Biotechnology*. 16, 1890–1896.

Lee, H. S., Seo, M.W., Kim, Z.H. and Lee, C.G. (2006). Determining the best specific light uptake rates for the lumostatic cultures in bubble column photobioreactors. *Enzyme and Microbial Technology*. 39, 447–452.

Lee, J.Y., Yoo, C., Jun, S.Y. Ahn, C.Y. and Oh, H.M. (2010). Comparison of several methods for effective lipid extraction from microalgae. *Bioresoure Technology*. 101, S75– S77.

Levine, R.B., Pinnarat, T. and Savage, P.E., (2010). Biodiesel production from wet algal biomass through *in situ* lipid hydrolysis and supercritical transesterification. *Energy Fuel*. 24, 5235-5243.

Lewis, T., Nichols, P.D. and McMeekin, T.A. (2000). Evaluation of extraction methods for recovery of fatty acids from lipid-producing microheterotrophs. *Journal Microbiology Methods*. 43, 107-116.

Li, X., Moellering, E.R., Liu, B., Johnny, C., Fedewa, M., Sears, B.B., Kuo, M.H. and Benning, C. (2012). A galactoglycerolipid lipase is required for triacylglycerol accumulation and survival following nitrogen deprivation in *Chlamydomonas reinhardtii*. *Plant Cell*. 24, 4670–4686.

Li, Y. and Qin, J.G. (2005). Comparison of growth and lipid content in three *Botryococcus braunii* strains. *Journal of Applied Phycology*. 17, 551–556.

Li, Y., Horsman, Wu, M., Lan, N. and Dubois, C.N. (2008). Biofuels from microalgae. *Biotechnology Progress*. 24, 815–820.

Lidstrom, P., Tierney, J., Wathey, B. and Westman, J. (2001). Microwave assisted organic synthesis-a review. *Tetrahedron*. 57, 9225–9283.

Liu, X., He, H., Wang, Y., Zhu, S. and Piao, X. (2008). Transesterification of soybean oil to biodiesel using CaO as a solid base catalyst. *Fuel*. 87, 216–221.

Lopez, E.J.A., Voltolina, D., Cordero, E. B. and Nieves, M. (2003). Produccion comercial de larvas de camaron y microalgas en cuatro estados de la Republica Mexicana *Biotechnical*. 5, 42–50.

Lotero, E., Liu, Y., Lopez, D. E., Suwannakarn, K., Bruce, D. A., & Goodwin Jr., J. G. (2005). Synthesis of biodiesel via acid catalysis. *Industrial & Engineering Chemistry Research*. 44(14), 5353-5363.

Lou, W., Zong, M. and Duan, Z. (2008). Efficient production of biodiesel from high free fatty acid-containing waste oils using various carbohydrate-derived solid acid catalysts. *Bioresource Technology*. 99, 8752–8758.

Loupy, A. (2004). Solvent-free microwave organic synthesis as an efficient procedure for green chemistry. *Actual Chim*. 272, 18–26.

Lucchesi, M.E., Chemat, F. and Smadja, J. (2004). Solvent-free microwave extraction of essential oil from aromatic herbs: comparison with conventional hydrodistillation. *Journal Chromatography A*. 1043, 323–327.

Luque, C.M.D. and Garcia, A.L.E. (1998). Soxhlet extraction of solid matrices: an outdated technique with a promising innovative future. *Analytical Chimica Acta*. 369, 1–10.

Ma, F. and Hanna, MA. (1999). Biodiesel production: a review. *Bioresource Technology*. 70, 1–15.

Macias, S.M.D, Mantell, C., Rodriguez, M., Martinez, D.L.O.E. and Lubian, L.M.M.O. (2005). Supercritical fluid extraction of carotenoids and chlorophyll a from *Nannochloropsis gaditana*. *Journal Food Engineering*. 66, 245–251.

Manirakiza, P., Covaci, A. and Schepens, P. (2001). Comparative Study on Total Lipid Determination using Soxhlet, Roese-Gottlieb, Bligh & Dyer, and Modified Bligh & Dyer Extraction Methods. *Journal of Food Composition and Analysis*. 14, 93-100

Makridis, P., Costa, R.A. and Dinis, M.T. (2006). Microbial conditions and antimicrobial activity in cultures of two microalgae species, *Tetraselmis chuii* and *Chlorella minutissima* and effect on bacterial load of enriched *Artemia metanauplii*. *Aquaculture*. 255, 76–81.

Masojidek, J. and Torzillo, G. (2008). Mass cultivation of fresh water microalgae. In: *Encyclopedia of ecology*. (pp. 2226–2235). United Kingdom: Oxford.

Meher, L.C., Sagar, D.V. and Naik, S.N. (2006). Technical aspects of biodiesel production by transesterification – a review. *Renewable Sustain Energy Review*. 10, 248–268.

Merchant, S.S., Prochnik, S.E., Vallon, O., Harris, E.H., Karpowicz, S.J. and Witman, G.B. (2007). The *Chlamydomonas* genome reveals the evolution of key animal and plant functions. *Science*. 318, 245–250.

Metting, F. B. (1996). Biodiversity and application of microalgae. *Journal of Industrial Microbiology & Biotechnology*. 17(5), 477-489.

Michael, Y. R., Stephen, P. S., Raymond, J.G. L., John, G. D., Elanor, M. B. and Michele, S. S., (2013). Effect of temperature and nutrient regimes on biomass and lipid production by six oleaginous microalgae in batch culture employing a two-phase cultivation strategy. *Bioresource Technology*. 129, 439-449.

Mittelbach, M. (1996). Diesel Fuel Derived from Vegetable Oils. VI Specifications and Quality Control of Biodiesel. *Bioresource Technology*. 56:7–11.

Molina, G.E., Sknchez, P.J.A., Garcia, C.F., Robles, M.A., Gimknez, A.G. and Lopez, A. D. (1995). The Production of Polyunsaturated Fatty Acids by Microalgae: from Strain Selection to Product Purification. *Process Biochemistry*. 30, 71 l-719.

Molina Grima, E., Belarbi, E.H., Acién Fernández, F.G., Robles Medina A., Chisti, Y. (2003). Recovery of microalgal biomass and metabolites: process options and economics. *Biotechnology Advance*. 20, 491–515.

Molina, E., Fernandez, J., Acien, G.G. and Chisti, Y. (2001). Tubular photobioreactor design for algal cultures. *Journal Biotechnology*. 92, 113–131.

Morrison, W.R. and Conventry, A.M. (1989). Solvent extraction of fatty acids from amylase inclusion complexes. *Starch*. 4, 24–27.

Muller, F. A., Moal, J. and Kaas, R. (2003). The Microalgae of Aquaculture. In: Støttrup, J.G.; McEvoy, L.A. (eds.): *Live Feeds in Marine Aquaculture* (pp. 206–252). United Kingdom: Oxford.

Mutanda, T., Ramesh, D., Karthikevan, S., Kumari, S., Anandraj, A. and Bux, F., (2011). Bioprospecting for hyper-lipid producing microalgal strains for sustainable biofuel production. *Bioresoure Technology*. 102, 57–70.

Naim, R., Muhammad, S.U.R, Madeha, S., Tariq, M. and Jong I. H. (2014). Current status, issues and developments in microalgae derived biodiesel production. *Renewable and Sustainable Energy Reviews*. 40, 760–778.

Nanton, D.A., Vegusdal, A., Rørå, A.M.B., Ruyter, B., Baeverfjord, G. and Torstensen, B.E. (2007). Muscle lipid storage pattern, composition, and adipocyte distribution in different parts of Atlantic salmon (*Salmo salar*) fed fish oil and vegetable oil. *Aquaculture*. 265(1), 230-243.

Nicholas, E., Leadbeater, S. J. P., Erik, S. and Victoria, A. W. (2005). An assessment of the technique of simultaneous cooling in conjunction with microwave heating for organic synthesis. *Tetrahedron*. 61, 3565–3585.

Nuchter, M., Muller, U., Ondruschka, B. and Tied, W.A.L. (2003). Microwave Assisted Chemical Reactions. *Chemistry Engineering Technology*. 26, 1207–1216.

Oh, S.H.,Kwon,M.C.,Choi,W.Y.,Seo,Y.C.,Kim,G.B. (2010).Long-term outdoor cultivation by perfusing spent medium for biodiesel production from *Chlorella minutissima*. *Journal Bioscience Bioengeenering.*110, 194–200.

Olivera, S.S., Lazic, M.L., Todorovic′, Z.B., Veljkovic′, V.B. and Skala, D.U. (2007). The effect of agitation intensity on alkali-catalyzed methanolysis of sunflower oil. *Bioresource Technology*. 98, 2688–2699.

Olufemi, A. and Stephen, K. L. O (2013). Optimization of two-step transesterification production of biodiesel from neem (*Azadirachta indica*) oil. *International Journal of Energy and Environmental Engineering*. 4, 39.

Owen, N.A., Inderwildi, O.R. and King, D.A. (2010). The status of conventional world oil reserves-hype or cause for concern. *Energy Policy*. 38, 4743–4749.

Parmar, A., Singh, N.K., Pandey, A., Gnansounou, E. and Madamwar, D. (2011). Cyanobacteria and microalgae: a positive prospect for biofuels. *Bioresource Technology*. 102, 10163–10172.

Pan, X., Niu, G. and Liu, H. (2002). Comparison of microwave-assisted extraction and conventional extraction techniques for the extraction of tanshinones from *Salvia miltiorrhiza bung*. *Biochemistry Engineering Journal.* 12, 71–77.

Pancha, I., Chokshi, K., George, B., Ghosh, T., Paliwal, C., Maurya, R. and Mishra, S. (2014). Nitrogen stress triggered biochemical and morphological changes in the microalgae *Scenedesmus* sp. CCNM 1077. *Bioresource Technogy*. 156, 146–154.

Patil, P.D., Gude, V.G., Mannarswamy, A., Deng, S., Cooke, P., Munson, M.S., Rhodes, I., Lammers, P. and Nirmalakhandan, N. (2010). Optimization of direct conversion of wet algae to biodiesel under super critical methanol conditions. *Bioresoure Technology*. 102, 118-122

Patil, P.D., Gude, V.G., Mannarswamy, A., Cooke, P., Munson, M.S. and Nirmalakhandan, N. (2011). Optimization of microwave-assisted transesterification of dry algal biomass using response surface methodology. *Bioresource Technology*. 102, 1399–1405.

Pernet, F. and Tremblay, R. (2003). Effect of ultrasonication and grinding on the determination of lipid class content of microalgae harvested on filters. *Lipids*. 38, 1191–1195.

Pierre, W., Greg, H., Bill, H., William, C. D., Helmut, K., Mahesh, B., Liang, Y. and Shulin C., (2014). Isolation, characterization, and validation of oleaginous, multi-trophic, and haloalkaline-tolerant microalgae for two-stage cultivation. *Algal Research*. 4, 2-11.

Popoola, T.O.S. and Yangomodou, O.D. (2006). Extraction, properties and utilization potentials of cassava seed oil. *Biotechnology*. 5, 38–41.

Prafulla, D.P., Veera, G.G., Aravind. M., Peter, C., Nagamany, N., Peter, L. and Shuguang, D. (2012). Comparison of direct transesterification of algal biomass under supercritical methanol and microwave irradiation conditions. *Fuel*. 97, 822–831.

Prafulla, D.P., Veera, G.G., Aravind. M., Peter, C., Stuart, M. M., Nagamany, N., Peter, L. and Shuguang, D. (2011). Optimization of microwave-assisted transesterification of dry algal biomass using response surface methodology. *Bioresource Technology*. 102, 1399–1405

Pratoomyot, J., Srivilas, P. and Noiraksar, T. (2005). Fatty acids composition of 10 microalgal species. *Journal Science Technology*. 27, 1179–1187.

Priego, C.F. and Luque, d.C.M.D. (2005). Focused microwave-assisted Soxhlet extraction: a convincing alternative for total fat isolation from bakery products. *Talanta*. 65, 98–103.

Pultz (2001). Photobioreactors: production systems for phototrophic microorganisms. *Applied Microbiology and Biotechnology*. 57, 287-293.

Qian, W.W., Yang, L. and Lu, X.P. (2008). Preparation of biodiesel catalyzed by KF/CaO with ultrasonic. In: Zhuang X, editor. *International Conference on Biomass Energy Technologies*. Guangzhou, China.

Raeesossadati, M.J., Ahmadzadeh H., McHenry M.P. and Moheimani, N.R. (2014). CO_2 bioremediation by microalgae in photobioreactors: Impacts of biomass and CO_2 concentrations, light, and temperature. *Algal Research.* 6, 78–85.

Ragni, M., Airs, R.L., Leonardos, N. and Geider, R.J. (2008). Photoinhibition of PSII in *Emiliania huxleyi* (Haptophyta) under high light stress: the roles of photoacclimation, photoprotection, and photorepair. *Journal of Phycology.* 44, 670–683.

Ramos, M.J., Fernández, C.M., Casas, A., Rodríguez, L., Pérez, Á. (2009). Influence of fatty acid composition of raw materials on biodiesel properties. *Bioresource Technology.* 100 (1), 261–268.

Ranjan, A., Patil, C. and Moholkar S.V. (2010). Mechanistic assessment of microalgal lipid extraction, *Indian Engineering Chemistry Research.* 49, 2979–2985.

Raven, J. and Geider, R. (1988). Temperature and algal growth. *New Phytology.* 110, 441-461.

Rawat, I., Ranjith Kumar, R., Mutanda, T. and Bux, F. (2011). Dual role of microalgae: phycoremediation of domestic waste water and biomass production for sustainable biofuels production. *Applied Energy.* 88, 3411–3424.

REN21. (Renewable *Energy Policy* Network for the 21st Century, 2008). Renewables 2007 Global Status Report. REN21: Paris.

Renaud, S. and Parry, D. (1994). Microalgae for use in tropical aquaculture effect of salinity on growth, gross chemical-composition and fatty-acid composition of species of marine microalgae. *Journal of Applied Phycology.* 6, 347–356.

Renaud, S., Parry, D., Thinh, L., Kuo, C., Padovan, A. and Sammy, N. (1991). Effect of light intensity on the proximate biochemical and fatty acid composition of *Isochrysis* sp. and *Nannochloropsis oculata* for use in tropical aquaculture. *Journal of Applied Phycology.* 3, 43–53.

Renaud, S.M., Thinh, L.V., Lambrinidis, G. and Parry, D.L. (2002). Effect of temperature on growth, chemical composition and fatty acid composition of tropical Australian microalgae grown in batch cultures. *Aquaculture.* 211, 195-214.

Richmond, A., (2000). Microalgal biotechnology at the turn of the millenium: a personal view. *Journal of Applied Phycology.* 12, 441–451.

Richmond, A, Cheng, W.Z. (2001). Optimization of a flat plate glass reactor for mass production of *Nannochloropsis sp.* outdoors. *Journal Biotechnology.* 85, 259–269.

Riekhof, W.R. and Benning, C. (2009) Glycerolipid biosynthesis. *In The Chlamydomonas sourcebook: Organellar and Metabolic Processes* (2nd edn) (Stern, D., ed.) (pp. 41–68). Elsevier

Rinchard, J., Czesny, S. and Dabrowski, K. (2007). Influence of lipid class and fatty acid deficiency on survival, growth, and fatty acid composition in rainbow trout juveniles. *Aquaculture* 264(1-4), 363-371.

Rittmann, B.E. (2008). Opportunities for renewable bioenergy using microorganisms. *Biotechnology Bioengineering.* 100, 203–212.

Rodolfi L., Chini Zittelli, G., Bassi, N., Padovani, G., Biondi, N., Bonini, G. and Tredici, M.R. (2009). Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. *Biotechnology Bioengineering.* 102, 100–112.

Roscher, E., Zetsche, K., 1986. The effects of light quality and intensity on the synthesis of ribulose-1,5-bisphosphate carboxylase andits mRNAs in the green alga *Chlorogonium elongatum. Plantation.* 167, 582–586.

Ruyters, G., (1984). Effects of blue light on enzymes. Blue light effects in biological system. *Proceeding Life Science.* 283–301.

Ryckebosch, E., Myuylaert, K. and Foubert, I. (2012). Optimisation of an analytical procedure for extraction of lipids from microalgae. *Journal of American Oil Chemists' Society.* 89, 189-198.

Saiban, S. and Brown, T.C. (1997). Kinetic model for cloud-point blending of diesel fuels. *Fuel.* 76(14), 1417–1423.

Sanchez, J.L.G., Berenguel, M., Rodriguez, F., Savilla. J.M.F., Alias, C.B. and Fernandez, F.G.A. (2003). Minimization of carbon losses in pilot-scale outdoor photobioreactors by model-based predictive control. *Biotechnology Bioengineering.* 84(5), 533–543.

Sarin, A., Arora, R., Singh, N.P., Sarin, R., Malhotra, R.K. and Kundu, K. (2009). Effect of blends of Palm–Jatropha–Pongamia biodiesels on cloud point and pour point. *Energy.* 34, 2016–2021.

Sastry, G.S.R., Krishna Murthy, A.S.R., Ravi Prasad, P., Bhuvaneswari, K. and Ravi, P.V. (2006) Identification and determination of bio-diesel in Diesel. *Energy Sources Part A.* 28, 1337–1342.

Schenk, P.M., Thomas-Hall, S.R., Stephens, E., Marx, U.C., Mussgnug, J.H., Posten, C., Kruse, O. and Hankamer, B. (2008). Second generation biofuels: high-efficiency microalgae for biodiesel production. *Bioenergy Research.* 1 (1), 20–43.

Schultz, I.R. and Hayton, W.L. (1994). Body size and the toxicokinetics of Trifluralin in rainbow trout. *Toxicology and Applied Pharmacology.* 129(1), 138-145.

Searchinger, T., Heimlich, R., Houghton, R.A., Dong, F., Elobeid, A. and Fabiosa, J. (2008). Use of U.S. croplands for biofuels increases greenhouse gases through emissions from land-use change. *Science.* 319(5867), 1238–1240.

Schuchardt, U, Ricardo Sercheli, R. and Vargas, R.M. (1998). Transesterification of vegetable oils: a review. *Journal Brazil Chemistry Society.* 9, 199–210.

Scott, S.A., Davey, M.P., Dennis, J.S., Horst, I., Howe, C.J., Lea-Smith, D.J. and Smith, A.G. (2010). Biodiesel from algae: challenges and prospects. *Current Opinion Biotechnology.* 21, 277–286.

Serrato, AG. (1981). Extraction of oil from soybeans. *Journal of the American Oil Chemists' Sociecty.* 58, 157–159.

Sharma, R., Chisti, Y. and Banerjee, U C. (2001) Production, purification, characterization and applications of lipases. *Biotechnology Advance.* 19, 627-662.

Shimada, Y., Watanabe, Y., Sugihara, A. and Tominaga, Y. (2002). Enzymatic alcoholysis for biodiesel fuel production and application of the reaction to oil processing. *Journal of the Molecular Catalysis B: Enzymatic.* 17, 133–142.

Show, K.Y. and Lee, D.J. (2014). Algal biomass harvesting. In: Pandey A ,Lee D.J., Chisti,Y., Soccol, C.R., editors. *Biofuels from Algae* (pp. 85–110). Burlington: Elsevier.

Shu, C.H., Tsai, C.H., Liao, W.H., Chen, K.Y. and Huang, H.C., (2012). Effects of light quality on the accumulation of oil in a mixed culture of *Chlorella* sp. and *S. Cerevisiae*. *Journal of Chemical Technology and Biotechnology.* 87, 601–607.

Shu, I. and Lee, C. (2003). Photobioreactor engineering: design and performance. *Biotechnology and Bioprocess Engeenering.* 8, 313–321.

Shuit, S.H., Lee, K.T., Kamaruddin, A.H. and Yusup, S. (2010). Reactive extraction of Jatropha curcas L. seed for production of biodiesel: process optimization study. *Environmental Science Technology.* 44, 4361–4367.

Shweta, S, Sharma, S. and Gupta, M.N. (2003). Enzymatic transestrification for biodiesel production. *Indian Journal Biochemistry Biophysiology.* 40, 392-399.

Singh, J., Gu, S., (2010). Commercialization potential of microalgae for biofuels production. *Renewable Sustainable Energy Review.*14 (9), 2596–2610.

Smith, B.T. and Davis, R.H. (2012). Sedimentation of algae flocculated using naturally-available, magnesium-based flocculants. *Algal Research.* 1, 32–39.

Song, D., Fu, J. and Shi, D. (2008). Exploitation of oil-bearing microalgae for biodiesel. *China Journal Biotechnol*ogy. 24, 341–348.

Spolaore. P., Joannis, C., Duran, E. and Isambert, A. (2006). Commercial application of microalgae. *Journal Bioscience Bioengeering.* 101, 87-96.

Suh, I.S. and Lee, S.B. (2001). Cultivation of a cyanobacterium in an internally radiating air-lift photobioreactor. *Journal of Applied Phycology.* 13, 381–388.

Sukenik, A., (1999). Production of eicosapentaenoic acid by the marine eustigmatophyte *Nannochloropsis*. Indian Cohen, Z. (Ed.), *Chemicals from microalgae*. (pp. 42-56) London: Taylor and Francis Ltd.

Sung, H. O., Min, C. K., Woon, Y. C., Yong, C. S., Ga. B. K., Do, H. K., Shin, Y. L., and Hyeon, Y. L. (2010). Long-term outdoor cultivation by perfusing spent medium for biodiesel production from Chlorella minutissima *Journal of Bioscience and Bioengineering*. 2, 194–200.

Suppalakpanya, K., Ratanawilai, S.B. and Tongurai, C. (2010). Production of ethyl ester from crude palm oil by two-step reaction with a microwave system. *Fuel*. 89, 2140–2144.

Talebi, A.F., Tabatabaei, M., Mohtashami, S.K., Tohidfar, M. and Moradi, F. (2013). Comparative salt stress study on intracellular ion concentration in marine and salt-adapted freshwater strains of microalgae. *Notulae Scientia Biologicae*. 5, 309–315.

Teo, C. L., Jamaluddin, H., Zain, N.A.M. and Idris, A, (2014). Biodiesel production via lipase catalysed transesterification of microalgae lipids from *Tetraselmis* sp. *Renewable Energy*. 68, 1-5.

Teo, C.L. and Idris, A., (2014). Enhancing the various solvent extraction method via microwave irradiation for extraction of lipids from marine microalgae in biodiesel production. *Bioresource Technology*. 171, 477-481.

Teo, C. L., Lai, L. W. and Idris, A., (2013). Comparison of Walne and F/2 medium in cultivating *Tetraselmis* sp. and *Nannochloropsis* sp. for biomass and lipid production (pp. 43-49). United States: DEStech Publicat Inc.

Tredici, M.R. (2007). Mass production of microalgae: photobioreactors, in: A. Richmond (Ed.), *Handbook of Microalgal Culture* (4th ed) (pp. 178–214). Oxford: Blackwell Publishing.

Tredici, M.R. (2010). Photobiology of microalgae mass cultures: understanding the tools for the next green revolution. *Biofuels*. 1, 143–162.

Uduman, N., Qi, Y., Danquah, M.K., Forde, G.M. and Hoadley, A. Dewatering of microalgal cultures: a major bottle neck to algae-based fuels. *Journal of Renewable Sustainable Energy*. 2, 012701–012715.

Ugwu, C.U., Ogbonna, J.C., Tanaka, H. (2003). Design of static mixers for inclined tubular photobioreactors. *Journal of Applied Phycology*. 15, 217–223.

Ugwu, C.U., Aoyagi, H. and Uchiyama, H. (2007). Influence of irradiance, dissolved oxygen concentration, and temperature onthe growth of *Chlorella sorokiniana*. *Photosynthetica*. 45, 309–311.

Ugwu, C.U., Aoyagi, H., Uchiyama, H. (2008). Photobioreactors for mass cultivation of algae. *Bioresource Technology*. 99, 4021–4028.

Umakanta, J., Nisha, V., Senthil, C. and Das, K.C., (2011). Evaluation of microalgae cultivation using recovered aqueous co-product from thermochemical liquefaction of algal biomass *Bioresource Technology*. 102, 3380–3387.

Vandamme, D., Foubert, I., Fraeye, I., Meesschaert, B. and Muylaert, K. (2012). Flocculation of *Chlorella vulgaris* induced by high pH: role of magnesium and calcium and practical implications. *Bioresource Technology*. 105, 114–119.

Venkataraman, N.S. (2002). editor. Focus on bio-diesel. *Nandini Chemistry Journal*. 9(IX), 19–21.

Vikram, K., Muthusivaramapandian, M., Basavaraj, P., Aloke, K. G. and Debasish, D. (2014). Evaluation and optimization of two stage sequential *in situ* transesterification process for fatty acid methyl ester quantification from microalgae. *Renewable Energy* 68, 560-569.

Virot, M., Tomaoa, V., Colnagui, G., Visinoni, F. and Chemata, F. (2007). New microwave integrated Soxhlet extraction, an advantageous tool for the extraction of lipids from food products. *Journal of Chromatography A*. 1174, 138–144.

Virot, M., Tomaoa, V., Colnagui. G., Visinoni, F. and Chemata, F. (2008). Microwave-integrated extraction of total fats and oils. *Journal of Chromatography A*. 1196–1197, 57–64.

Volkman, J. K, JeVrey, S. W., Nichols, P. D., Rogers, G. I. and Garland, C. D. (1989). Fatty acid and lipid composition of 10 species of microalgae used in mariculture. *Journal of Experimental Marine Biology and Ecology*. 128, 219–240.

Wahal, S. and Viamajala, S. (2010). Maximizing algal growth in batch reactors using sequential change in light intensity. *Applied Biochemistry and Biotechnology*. 161, 511–522.

Wahidin, S., Idris, A, and Shaleh, S.R.M. (2013). The influence of light intensity and photoperiod on the growth and lipid content of microalgae *Nannochloropsis* sp. *Bioresource Technology*. 129, 7–11.

Wahidin, S., Idris, A. and Siti, R. M. S. (2014). Rapid biodiesel production using wet microalgae via microwave Irradiation. *Energy Conversation Management*. 84, 227–233.

Wahlen, B.D., Willis, R.M., and Seefeldt, L.C. (2011). Biodiesel production by simultaneous extraction and conversion of total lipids from microalgae, cyanobacteria, and wild mixedcultures. *Bioresource Technology*. 102(3), 2724–2730.

Wang, C.Y., Fu, C.C. and Liu, Y.C. (2007). Effects of using light-emitting diodes on the cultivation of *Spirulina platensis*. *Biochemistry Engineering.* J. 37, 21–25.

Ward, B., Maria, C., René, H.W. and Marcel, J. (2013). Cultivation of microalgae on artificial light comes at a cost. *Algal Research.* 2, 333–340.

Watanabe, Y. and Saiki, H. (1996). Development of a photobioreactor incorporating *Chlorella* sp. for removal of CO_2 in stack gas. *Energy Conversation Management.* 6–9, 721–724.

Williams, P.J. (2007). Biofuel: microalgae cut the social and ecological costs. *Nature.* 450, 478.

Wiltshire, K.H., Boersma, M.M.A. and Buhtz, H. (2000). Extraction of pigments and fatty acids from the green alga *Scenedesmus obliquus (Chlorophyceae)*. *Aquatic Ecology.* 34, 119–126.

Widenfalk, A., Bertilsson, S., Sundh, I. and Goedkoop, W. (2008). Effects of pesticides on community composition and activity of sediment microbes – response at various levels of microbial community organization. *Environmental Pollution.* 152(3), 576-584.

Xin, D., Zhen, F. and Yun, H. L. (2010). Ultrasonic transesterification of Jatropha curcas L. oil to biodiesel by a two-step. *Process Energy Conversion and Management.* 51, 2802–2807.

Xiou, J.Y. and Xu, Y. (2005). Analysis of lysophospholipids in human body fluids: comparison of the lysophospolipid content in malignant vs non-malignant disease. In: Feng Li, Glen D, editors. *Functional lipidomics. CRC Press.* pp 125.

Yakata, N., Sudo, Y. and Tadokoro, H. (2006). Influence of dispersants on bioconcentration factors of seven organic compounds with different lipophilicities and structures. *Chemosphere.* 64(11), 1885-1891.

Yangmin, G. and Mulan, J. (2011). Biodiesel production with microalgae as feedstock from strains to biodiesel. *Biotechnolology Letters.* 33, 1269–1284.

Yeh, N.C. and Chung, J.P. (2009). High-brightness LEDs-energy efficient lighting sources and their potential in indoor plant cultivation. *Renewable Sustainable Energy Review.* 13, 2175–2180.

Yoon, J.H., Shin, J.H., Ahn, E.K. and Park, T.H. (2008). High cell density culture of Anabaena variabilis with controlled light intensity and nutrient supply. *Journal of Microbiology and Biotechnology.* 18, 918–925.

Yue, Z., Wing, T. W. and Ka, F. Y. (2013). One-step production of biodiesel from rice bran oil catalyzedby chlorosulfonic acid modified zirconia via simultaneous esterification and transesterification . *Bioresource Technology.* 47, 59-64.

Yun, M.S., Lee, S.H. and Chung, I.K. (2010). Photosynthetic activity of benthic diatoms in response to different temperatures. *Journal of Applied. Phycology*. 22, 559–562.

Zayed, A.H. and Jehad, Y. (2014). Parametric study of the alkali catalyzed transesterification of waste frying oil for Biodiesel production. *Energy Conversation Management*. 79, 246–254.

Zenouzi, A., Ghobadian, B., Hejazi, M. and Rahnemoon, P. (2013). Harvesting of microalgae *Dunaliella salina* using electroflocculation. *Journal of Agriculture Science Technology*. 15, 879–888.

Zhang, Q., Wu, X., Xue, S., Liang, K., Cong, W. (2012). Study of hydrodynamic characteristics in tubular photobioreactors. *Bioprocess Biosysthesis Engineering*. 36, 143–150.

Zhang, S., Zu, Y.G., Fu, Y.J., Luo, M., Zhang, D.Y. and Efferth, T. (2010). Rapid microwave-assisted transesterification of yellow horn oil to biodiesel using a heteropolyacid solid catalyst. *Bioresource Technology*. 101, 931-936.

Zhou, S.J. and Hawley, M.C. (2003). A study of Microwave Reaction Rate Enhancement Effect in Adhesive Bonding of Polymers and Composites. *Composition Structure*. 61, 303–309.

Zhou, W., Min, M., Hu, B., Ma, X., Liu, Y. and Wang, Q. (2013). Filamentous fungi assisted bio-flocculation: a novel alternative technique for harvesting heterotrophic and autotrophic microalgal cells. *Seperation and Purification Technology*. 107, 158–165.