CULTIVATION OF *NANNOCHLOROPSIS* SP. FOR BIODIESEL PRODUCTION VIA MICROWAVE IRRADIATION DIRECT TRANSESTERIFICATION USING IONIC LIQUID

SUZANA BT WAHIDIN

A thesis submitted in fulfilment of the
requirements for the award of the degree of
Doctor Philosophy (Bioprocess Engineering)

Faculty of Chemical Engineering
Universiti Teknologi Malaysia

MAY 2015

To my beloved late father and mother,
Mr Wahidin Majid & Mrs Rohani Harun,
My lovely hubby,
Mohd Ridzuan Mohd Noor,
My cute Prince,
Muhammad Daryus Zaffran,

with my eternal love and thank you for being the best thing that ever happened to me

ACKNOWLEDGEMENTS

Foremost, I would like to thank Allah almighty for providing me this opportunity and all His guidance that make my PhD thesis become realism.

I would like to express my sincere gratitude to my main supervisor, Prof. Dr. Ani Idris for the continuous support of my PhD study and research, for her patience, motivation, enthusiasm, and immense knowledge. Her guidance helped me in all the time of research and writing of this thesis. I could not have imagined having a better advisor and mentor for my PhD study.

I would like to express my deepest gratitude to my co-supervisor, Associate Prof. Dr. Sitti Raehanah Muhamad Shaleh (Universiti Malaysia Sabah) for her excellent guidance on microalgae cultivation and providing me with microalgae strains for doing this research. I want to express my deep thanks to Prof. Dr. Noordin Mohd. Yusof for diligently assisting me in carrying out all statistical analysis under his constructive comments. The greatest appreciation goes to all the technicians at the Faculty of Chemical engineering, Faculty of Mechanical Engineering and Faculty of Electrical Engineering for their assistance on different technical areas of the project and gives full cooperation, especially Mr. Yaakob, Mrs. Siti Zalita, Mr. Noordin, Mr. Jeffri, Mr. Mohammed and Mr. Yahya. Special thanks also goes to Ms. Lusia at Borneo Marine Research Institute, Universiti Malaysa Sabah for providing the training programme for cultivation of microalgae culture and assistance in providing me the microalgae strains. I truly appreciate all of the assistance that each of you had provided me, whether they were administrative or technical.

My beloved husband, Mohd Ridzuan Mohd. Noor, without your supports and encouragements, I could not have finished this work, it was you who kept the fundamental of our family, and I understand it was difficult for you, therefore, I can just say thanks for everything and may Allah give you all the best in return. I want to express my gratitude and deepest appreciation to my lovely son, Daryus Zaffran, for his great patience and understandings and for being a nice boy. Without their support, this opportunity would never have been possible. I would like to express my special appreciation to my late father, Mr. Wahidin Majid, and my lovely mum, Mrs. Rohani Harun, as well as my wonderful siblings, my brothers and sisters. They were always supporting me and encouraging me with their best wishes.

Sincere gratitude to all lab mates, Nursia, Nurdiana, Effaliza, Farahah, Raha, Asma', Hasrul and Jacky under Advanced Bioprocessing and Separation (ABS) research group who work together in Analysis Laboratory. I greatly appreciate your excellent assistance and your spiritual supports for me during my PhD study. I will never forget the time that we were together and discussed about research and being awesome friends.

Many thanks to my best friends Wan Nurhuesna, Izume Ayuna, Asimi Ana, Haniza, Nurul Nabihah, Dr. Ruzainah, Elmy Nahida and Dr. Anisah; thank of them for their support, great bonding, love and friendship. Our friendship provides me with great happiness and encourages me to complete PhD study.

Finally, I also wish to express my appreciation to countless individuals who have contributed directly or indirectly towards accomplishment of my research work.

SUZANA BINTI WAHIDIN, 2015

ABSTRACT

Nannochloropsis sp. has been recognized as a potential source of biomass feedstock for biodiesel due to their abilities to grow rapidly, high oil and lipid content. However major challenges faced are the expensive downstream processes such as dewatering and drying; utilisation of large volumes of solvent; and tedious extraction processes. The novelty of this study is the production of biodiesel from wet microalgae biomass via microwave irradiation direct transesterification (DT) process with ionic liquid as co-solvent system. *Nannochloropsis* sp. was cultivated aseptically for 9 days at different light intensities (50, 100 and 200 $\mu molm^{-2}\ s^{-1}$) and photoperiod cycles (24:0, 18:06 and 12:12 h light:dark) in a 5 L photobioreactor at 23 ^{o}C using F/2 and Walne's medium for maximum growth and lipid content. Microwave irradiation was used in the extraction of lipids from microalgae and also the transesterification and the results were compared with that obtained via conventional waterbath heating. The effect of microwave irradiation on microalgae cell morphology was then identified. The effects of parameters such as different types of solvent and microwave irradiation time on biodiesel yield were also investigated. A DT process was performed taking into account the important factors such as reaction temperature, reaction time, ratio of methanol to algae and ratio of ionic liquid to methanol. Results revealed that the maximum growth and lipid content were obtained under 100 $\mu molm^{-2}s^{-1}$ light intensity and photo period cycles (18:06 h) after 7 days cultivation using Walne's medium; achieving maximum cell concentration of 12.5×10^{7} $cell.ml^{-1}$, which corresponded to the growth rate of 0.453 d^{-1} and lipid content of 38.31 %. Microwave accelerates the disruption of microalgae cell walls resulting in rapid release of oil. The use of IL2 as co-solvents was found to be most effective in disrupting microalgae cell. It was seen that the cell disruption achieved a maximum of 99.73 % after 15 min of DT process. The mixture of methanol-IL2 extracted the lipid from microalgal biomass, concurrently transesterified the extracted lipids to produce biodiesel under microwave irradiation. The maximum (36.79 %) biodiesel yield was obtained in 15 min when IL2 was used as co-solvent. The biodiesel production via DT under microwave irradiation was optimized using Central Composite Design (CCD). The main effects considered were reaction time (5 – 25 min); wet algae to methanol ratio (1:4 – 1:12) and methanol:IL2 ratio (1:0.5 - 1:1); and the response variables measured was biodiesel yield. Statistical analysis revealed optimum biodiesel yield (40.9 %) was achieved at 15 min of reaction time, algae:methanol ratio of 1:4 and methanol:IL2 ratio of 1:0.5 (v/v). The major composition of fatty acid methyl esters (FAMEs) were oleic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:1n9c) and linoleic acid (C18:2n6c). In conclusion DT process using combination of methanol-IL2 coupled with microwave irradiation is a very attractive novel method to produce maximum biodiesel yield.

ABSTRAK

Nannochloropsis sp. telah diiktiraf sebagai sumber bahan mentah biojisim untuk menghasilkan biodiesel kerana kadar pertumbuhannya yang cepat, kandungan minyak dan lipid yang tinggi. Cabaran utama yang dihadapi adalah kos pemprosesan hiliran yang mahal seperti penyahairan dan pengeringan, penggunaan pelarut yang banyak. Novelti kajian ini adalah penghasilan biodiesel daripada biojisim mikroalga basah melalui penyinaran gelombang mikro dan proses transesterifikasi langsung (DT) dengan menggunakan cecair berion sebagai sistem sepelarut. *Nannochloropsis* sp. dikultur secara aseptik selama 9 hari pada keamatan cahaya berbeza (50, 100 dan 200 $\mu molm^{-2}s^{-1}$) dan kitaran fotokala (24:0, 18:06 dan 12:12 j cahaya:gelap) di dalam 5 L photobioreaktor pada suhu 23 ^{o}C menggunakan medium F/2 dan Walne untuk pertumbuhan dan kandungan lipid yang maksima. Sinaran gelombang mikro digunakan dalam pengekstrakan lipid dari mikroalga dan juga transesterifikasi dan data dibandingkan dengan pemanasan waterbath konvensional. Kesan parameter seperti jenis pelarut dan masa tindak balas daripada pendedahan kepada sinaran gelombang mikro kepada penghasilan biodiesel dikenalpasti. Proses DT dibuat dengan mengambil kira faktor yang penting seperti suhu dan masa tindakbalas, nisbah alga/methanol dan nisbah methanol/IL2. Keputusan menunjukan pertumbuhan dan kandungan lipid yang maksimum diperolehi pada keamatan cahaya 100 $\mu molm^{-2}s^{-1}$ dan foto kitaran 18:06 j selepas 7 hari pengkulturan di dalam media Walne; mencapai sel maksimum 12.5 x 10^{7} $sel.ml^{-1}$ dengan kadar pertumbuhan 0.453 d^{-1} dan kandungan lipid 38.31%. Gelombang mikro mempercepatkan lagi pemecahan dinding sel mikroalga dan menyebabkan minyak lebih cepat dikeluarkan. Penggunaan IL2 sebagai sistem sepelarut merupakan paling berkesan untuk memecahkan sel. Ianya dilihat dengan pemecahan sel yang maksimum sebanyak 99.73% dicapai selepas 15 min proses DT. Campuran metanol-IL2 menyarin lipid daripada biojisim mikroalga dan secara serentak lipid ditransesterifikasikan untuk menghasilkan biodiesel di bawah sinaran gelombang mikro. Penghasilan biodiesel yang maksimum telah dicapai pada 15 min apabila IL2 telah digunakan sebagai sepelarut (36.79 %). Penghasilan biodiesel menggunakan biojisim basah dalam proses DT telah dioptimumkan dengan menggunakan perisian *Central Composite Design (CCD).* Faktor-faktor utama yang diambil kira adalah masa tindak balas (5-25 min), nisbah alga/metanol (1:4-1:12) dan nisbah methanol/IL_2 (1:0.5-1:1), dan pembolehubah maklumbalas yang dikaji ialah penghasilan biodiesel. Analisis statistik menunjukkan penghasilan biodiesel yang maksimum (40.9%) telah dicapai pada 15 min masa tindakbalas, nisbah alga:metanol 1:4 dan metanol:IL2 nisbah 1:0.5. Komposisi utama asid lemak metil ester (FAMEs) adalah asid oleik (C16:0), asid palmitoleic (C16:1), asid stearik (C18:1n9c) dan asid linoleik (C18:2n6c). Kesimpulannya proses DT menggunakan kombinasi methanol:IL2 dibantu dengan sinaran gelombang mikro adalah satu kaedah baru yang amat menarik untuk menghasilkan biodiesel yang maksimum.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

β_o	-	Constant coefficient
2_k	-	Factorial (or fractional factorial)
$2k$	-	Axial or star runs
gL^{-1}	-	Concentration (gram per liter)
H_2SO_4	-	Sulphuric acid
HCl	-	Hydrochloric acid
mg	-	Mass, milligram
^oC	-	Temperature (Degree Celsius)
g	-	Mass, gram
v/v %	-	Volume per volume percentage
ε'	-	Dielectric constant
ρ	-	Density
α	-	Axial point
ε''	-	Dielectric loss
$\mu molm^{-2}s^{-1}$	-	Light intensity, micromole per meter square per second
μ	-	Specific growth rate
tan δ	-	Loss tangen
R^2	-	Linear regression constant
%	-	Percentage
Kv	-	Kilo volt
O_2	-	Oxygen
s	-	Time, second
KOH	-	Potassium hydroxide
sp.	-	Subspecies

μm	-	Distance, micrometer
mw	-	Microwave
CO_2	-	Carbon dioxide
min	-	Time, minute
m	-	Distance, meter
m^3	-	Volume, cubic meter
h	-	Time, hour
>	-	More than
mm	-	Distance, milimeter
wt%	-	Weight percentage
CH_3COCl	-	Acetyl chloride
cm	-	Distance, centimeter
M	-	Concentration, Molar
L	-	Volume, Liter
nm	-	Distance, nanometer
MHz	-	Megahertz
W/m^2	-	Power, Watt per meter square
% C_t	-	Cell disruption percentage
N_1	-	Cell number concentration at t_1
N_2	-	Cell number concentration at t_2
k	-	Division rate
W	-	Watt
f	-	Frequency
N_t	-	Average number intact cells at certain time
D	-	Distribution coefficient
P	-	Power dissipation density
Ei	-	Electric field strength
C_p	-	Specific heat
E	-	Total energy
HCO_3	-	Bicarbonate
w/w_{DW}	-	Weight per dry weight
$AlCl_3$	-	Aluminum chloride
BMIM-Cl	-	1-butyl-3-methyl imidazolium chloride
$[PF_6]$-	-	Hexafluorophosphate

$[BF_4]$-	-	Tetrafluoroborate,
$[CF_3SO_3]$-,	-	Trifluoromethane sulfonate or triflate
$[(CF_3SO_2)_2N]$-	-	Bis-(trifluoromethanesulfonyl) amide
$[NTf_2]$-	-	Bistriflamide
$[N(SO_2CF_3)_2]^-$	-	*N*-Phenyl-bis(trifluoromethanesulfonimide)
$[(CF_3SO_2)_3]$-	-	Ttris {(trifluoromethyl) sulfonyl} methanide
$[Ms_2N]$-	-	Bis (methanesulfonyl) amide
[R1R2IM] +	-	Alkylimidazolium
[RPy] +	-	Alkylpyridinium
[NR4] +	-	Tetraalkylammonium
[PR4] +	-	Tetraalkylphosphonuim
NaOH	-	Sodium hydroxide
SrO	-	Strontium oxide
ml	-	Volume, milliliter
vvm	-	Gas volume flow per unit of liquid volume per minute, volume per volume per minute
µL	-	Volume, microliter
$mgml^{-1}$	-	Milligram per milliliter
g	-	Mass, gram
HF	-	Hydrogen fluoride
$FeCl_3.6H_2O$	-	Iron (III) Chloride hexahydrate
$MnCl_2.4H_2O$	-	Manganous chloride
H_3BO_3	-	Boric acid
Na_2 EDTA	-	Disodium salt dehydrate
$NaH_2PO_4.2H_2O$	-	Sodium di-hydrogen orthophosphate
$NaNO_3$	-	Sodium nitrate
$ZnCl_2$	-	Zinc chloride
$CoCl_2.6H_2O$	-	Cobalt (II) chloride hexahydrate
$NaMoO_4.2H_2O$	-	Sodium molybdate dihydrate
$(NH_4)_6Mo_7O_{24}.4H_2O$	-	Ammonium molybdate tetrahydrate
$CuSO_4.5H_2O$	-	Copper (II) sulfate pentahydrate
NaCl	-	Sodium chloride,
C14:0	-	Myristic acid methyl ester
C16:0	-	Palmitic acid methyl ester

C16:1	-	Palmitoleic acid methyl ester
C17:0	-	Heptadecanoic Acid
C18:0	-	Stearic acid methyl ester
C18:1,*cis*-9	-	Oleic acid methyl ester
C18:1,*trans*-9	-	Elaidic acid methyl ester
C18:2,*cis*-9,12	-	Linoleic acid methyl ester
C18:2,*trans*-9,12	-	Linolelaidic acid methyl ester
C18:3,*cis*-9,12,15	-	Linolenic scid methyl ester
C20:0	-	Arachidic acid methyl ester
C22:0	-	Behenic acid methyl ester
$ZnSO_4 7H_2O$	-	Zinc Sulfate Heptahydrate

LIST OF ABBREVIATIONS

ANOVA	-	Analysis of variance
CCD	-	Central composite design
FESEM	-	Field emission scanning electron microscope
FCD	-	Face centered design
RSM	-	Response surface methodology
PFD	-	Photon flux density
GHGs	-	Greenhouse gas emissions
GF/C	-	Glass microfiber design
DT	-	Direct transesterification
FFA	-	Free fatty acid
SCM	-	Supercritical methanol
PE	-	Polyethylene
PVC	-	Polyvinyl chloride
TAG	-	Triglyceride
FID	-	Flame ionization detector
SEM	-	Scanning electron microscopy
ID	-	Internal diameter
ATP	-	Adenine tri-phosphate
NADPH	-	Nicotinamide adenine dinucleotide phosphate
L:D	-	Light:dark cycles
NR	-	Nile red
PTFE	-	Polytetrafluoroethylene
IL	-	Ionic liquid
TSIL	-	Task specific ionic liquid
SFE	-	Supercritical fluid extraction
FAMEs	-	Fatty acid methyl esters
mw	-	Microwave
PFA	-	Perfluoroalkoxy alkane

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Research Background

The concerns for greenhouse gas emissions (GHGs), an accelerated decrease in fossil fuels, steeply increase in crude oil prices and energy insecurity has attracted worldwide interest in sustainable and renewable energy sources (Cui *et al*., 2014; Owen *et al*., 2010; Soh and Zimmerman, 2011; Kaur *et al*., 2012; Li *et al*., 2011). In order to meet petro-diesel replacement, renewable energy sources should not only be environmentally beneficial but it should be producible, economical and costs competitive to give a meaningful impact on sustainable energy demands (Parmar *et al*., 2011; Budarin *et al*., 2012). Biodiesel fuel is one type of alternative renewable fuel, which is highly biodegradable, has minimal toxicity or non-toxic and environmentally friendly. Recently, biodiesel has been considered as one of the best sustainable sources of petroleum-based fuels (Man and Keat, 2012; Chisti, 2008).

Biodiesel is a fuel derived from the transesterification process which comprised of methyl-esters of fatty acids from animal fats and vegetable (crops) oils. Current production of biodiesel fuel includes oil of crops (palm oil, soybean, corn oil, rapeseed oil, coconut oil and sunflower oil), animal lards (fats) and used cooking oil. Unfortunately biodiesel produced from these feedstocks are not able to replace fossil fuels because they are regarded as competitors to food supply. The findings in biotechnology have proven that microalgae, yeast and fungi are potential raw materials for biodiesel production due to high amounts of lipid composition (Amaro *et al*., 2011).

Microalgae is regarded as an interesting sustainable feedstock for biodiesel manufacturing and has the potential to completely displace petrol-diesel fuels because of their rapid growth potential (microalgae biomass was higher 5-30 times of oil crops) and has high lipid content (Chisti, 2008). Chen *et al*. (2009) stated that microalgae can be grown without encroaching on arable land or cultivated in brackish water. Zhang *et al*. (2003) found that microalgae oil is mostly accumulated as triglycerides and can be transformed to biodiesel. Microalgae such as green algae have been found to contain proportionally high level of lipids. Different kinds of microalgae have different capability in producing lipids yields for lipid productivity, depending on types of microalgae species. Among the locally marine microalgae, *Nannochloropsis* sp. displayed the best potential biodiesel production because they have both high biomass productivity and high lipid content (Thi *et al*., 2011; Rodolfi *et al*., 2009; Chiu *et al*., 2009; Gouveia and Oliveira, 2009).

However, algal biodiesel production still faces hurdles due to the high cost associated with microalgae mass production, harvesting, dewatering and preparation for conventional lipid extraction and transesterification into biodiesel. Harvesting technology of microalgae biomass requires a universal separation application such as universal centrifuge and flocculant agents to recover microalgae mass. The major challenge for commercialized biodiesel production from microalgae is the high cost of recovering the oil from the microalgae prior to converting it into biodiesel. Numerous methods for extraction of lipids from microalgae have been applied such as solvent extraction (liquid-liquid extraction), supercritical fluid extraction (SFE) (Mouahid *et al*., 2013; Liau *et al*., 2010), ultrasonic extraction and mechanical pressing. Such extraction methods require longer extraction time, large volumes of solvent except using SFE and are energy-cost intensive (Patil *et al*., 2011; Kanithar, 2010; Um and kim, 2009; Boldor *et al*., 2010).

Lardon *et al*., (2009) analyzed the lipid extraction process and reported that 90% of energy consumed for biodiesel conversion from microalgae biomass are used for the lipid extraction. Thus, any improvement in microalgal oil productivity will give a major impact on lowering the costs of the extraction process. Some of microalgae species can accumulate significant quantities of triglycerides (20-50% of

total dry weight). Conversion of algal triglycerides to biodiesel production required the conventional extraction, where microalgal oil has been mixed with organic solvents such as chloroform, methanol and n-hexane). Xu *et al*. (2006) and Cheng *et al*. (2009) explain that the organic solvents in lipid extraction can be removed via distillation unit and triglycerides are then synthesized with alcohol (e.g. methanol) using either acid-catalyzed or base-catalyzed for biodiesel production.

Works by Demirbas (2009) have shown that supercritical transesterification is one of the alternative processes for produce biodiesel from microalgae lipid conversion. This process facilitated without the catalyst but required high reaction temperature (>240^{o}C) and pressure (> 8 MPa). Supercritical fluid extraction (SFE) has gained interest because it significantly lowers the requirement for organic solvents and thus takes into consideration the concerns for waste disposal after reaction. Ehimen *et al*., (2010) reported that SFE demonstrated the shortest reaction time (120-240 sec) to complete the reaction from lipid to biodiesel. SFE method required a simple purification step and also produce higher biodiesel yields compared to solvents extraction and soxhlet extraction processes. However, this method has several disadvantages due to the adverse process economics as well as safety concerns related to the reaction condition. Furthermore, sample size and water content conditions were affecting the SFE process and must be improved to achieve efficient extractions. Thus, the new extraction technologies which are efficient and cost effective on large scales must be explored and developed for microalgae systems.

Microwave technology has allowed the development of rapid, safe and cost-effective method for extracting lipids and does not require that samples be devoid of water. Performance of microwave lipid extraction was qualitatively (all lipid classes) and quantitatively comparable with that of the conventional Folch's method for various biological samples. There have limited articles in literature that mentioned the acceleration of lipid extraction from microalgae by microwave irradiation. The application of radio frequency microwave energy offers a fast and easy route to this valuable biofuel with the advantages of enhancing the reaction rate (2 min instead of 2 hr process reaction) and improving the separation techniques

(Shakinaz, 2010). The advantages of using microwave energy as a non-contact heat source for the extraction of analytes from plant materials include: more effective heating, faster energy transfer, reduced thermal gradients, selective heating, reduced equipment size, faster respond to process heating control, faster start-up, increased production and elimination of process steps (Matthieu, 2008).

Direct transesterification (DT) method can displace the conventional biodiesel production process which is reducing the fuel conversion processing units and costs associated. Unlike the conventional method where oil is first extracted, followed by the transesterification process of FAMEs. In this method, the oil is simultaneously extracted and transesterifed into methyl ester in-situ in one single process. Hence, separate extraction and oil refining steps can be eliminated since extraction is simultaneously carried out by reactant itself (Ehimen *et al*., 2010).

The single step transesterification (direct transesterification) method was performed on dried microalgae powder by normal heating at 90^{o}C for 40 minutes and reacted with alcohol and acid catalyzed (Johnson and Wen, 2009). Koberg *et al*., (2011) and Patil *et al*., (2012) performed direct transesterification on the dried samples using microwave radiation extraction and sonication extraction for biodiesel production. This biodiesel production scheme could therefore aid in the simplification of the fuel conversion process, potentially reducing the overall process cost, hence lowering the final fuel product costs as well. However, the health security and regulatory problems associated with the use of organic solvents should be addressed (Tsigie, *et al*., 2012). In addition, efficient solvent recovery processes are needed to commercialize these processes.

Environmental friendly concept has placed a renewed emphasis on careful solvent selection and search for less harmful alternatives. In the past, the emphasis in industrial chemistry was placed on product yield and quality. Increasingly, solvent use during manufacture is viewed in terms of avoiding the costs associated with disposal, legal liabilities and regulatory constraints. Over the years, our society has become more aware of the environmental problems. This awareness has led to interest in cleaner and greener technologies which reduce or even eliminate the use

of hazardous and toxic materials. As one of the most promising green technologies is application of green solvents and considered a category which includes supercritical CO_2 and microwave assisted techniques, aqueous biphasic systems and ionic liquids. Ionic liquids (ILs) have been described as "green" alternatives to organic solvents and have many unique physiochemical properties and are becoming more attractive substitutes. ILs are non-volatile, thermal stable solvents and non flammable (Jonathan *et al*., 2001). Normally the traditional organic solvents are volatile and are used in large amounts for complete reaction.

To the best of our knowledge, there have been no reports on the combined use of ionic liquid as green solvent in microwave system for biodiesel production technique in order to further reduce biodiesel production costs while increasing of biodiesel yields. Thus, in this study the wet biomass microalgae of *Nannochloropsis* sp. are converted to biodiesel using direct transesterification by microwave technique and ionic liquid as green solvent. Microwave accelerates the disruption of microalgae cells and as a result the oil are released rapidly from cell wall. Ionic liquid as a green solvent or catalyst can be filtered and reused as a solvent or catalyst.

In this study the effect and feasibility of using an ionic liquid-microwave extraction system for one stage direct transesterification of biodiesel production are evaluated since this technology has the potential to be introduced for industrial scale applications due to its high heating rates, scalability, lesser solvent requirements, continuous nature and environmentally friendly process.

1.2 Problem Statement

Amaro *et al*. (2011) reported that wet feedstock can be directly applied for microalgal oil production and should be an effective extraction technology. Since microalgae are cultivated in aqueous environments, removal of water towards a paste consistency-typically 10-30% (w/w_{DW}), is cost intensive because of the high latent heat of vaporization of water. Thus, one step transesterification can avoid some of

the energy intensive drying and lipid extraction process and can make the overall process more efficient.

The biodiesel production have been successfully produced using various extraction methods from microalgal oil (Koberg *et al*., 2011; Ranjan *et al*., 2010; Lee *et al*., 2010). Most of the biodiesel production method involved a two-step processing. The first step involves extraction of lipid using different kinds of extraction method such as solvent extraction (mixture of chloroform-methanol), soxhlet extraction (with n-hexane as solvent), supercritical fluid extraction or supercritical CO_2 extraction, ultrasound-assisted extraction and limited study using a microwave radiation technique. The microalgal oil was then converted to biodiesel through transesterification method with base-catalyzed or acid-catalyzed (Ross *et al*., 2010).

Since the conventional method (extraction-transesterification) is not costs competitive, a direct transesterification technique is another alternative for biodiesel production. A direct transesterification method involves only a single step process, where the microalgae biomass is extracted and converted to fatty acid methyl esters (FAMEs) or biodiesel. In this process, microalgae biomass would be mixed with large quantities of organic solvent and used either acid-catalyzed or base-catalyzed. The continued use of large quantities of organic solvents as liquid media and prolong reaction time of simultaneous extraction-transesterification for unsure complete reaction and recovery are the factors that hindered the use of the direct transesterification method.

In order to eliminate the use of toxic organic solvents (e.g. chloroform, n-hexane or benzene) and hazardous catalysts, ionic liquid is used to replace the solvents in the direct transesterification procedure with microwave heating. Thus, in this study a novel simultaneous extraction transesterification technique in explored using a combination of ionic liquids and microwave heating.

1.3 Objectives and Scope

The objective of this research is to produce biodiesel from wet biomass of *Nannochloropsis* sp. as a feedstock through the microwave simultaneous extraction-transesterification or direct transesterification (DT) using ionic liquid as a green solvent. In order to achieve this objective *Nannochloropsis* sp. is cultivated in a photobioreactor and the DT method is then compared with several conventional solvents. Among others the objectives are as follows:

1. To investigate the effect of different light intensities and different photoperiod regimes (exposure to light:dark cycles) on the growth of *Nannochloropsis* sp. and total lipid content.
2. To study the potential of producing biodiesel from wet microalgae biomass using different biodiesel preparation techniques (conventional water bath and microwave irradiation)
3. To investigate parameters such as different types of solvent and reaction time to microwave irradiation
4. To identify the microwave effect on microalgae cell and the percentage of disrupted cell.
5. To optimize direct transesterification process using design of experiment (DOE) where the one step direct transesterification process is performed taking into account all the important parameters such as reaction temperature and time, ratio of algae to methanol volume and ratio of ionic liquid to methanol volume.

1.4 Outline of the Thesis

The thesis is basically divided into six chapters. The research background in biodiesel production using microalgae is discussed in Chapter 1. This chapter also highlights the problem statement, research objectives and scope of the study. A comprehensive literature review were performed prior to any experimental work.

Literature review providing the state of art background to the research is provided in detail in Chapter 2. The topic highlights on microalgae, extraction process, microwave heating, biodiesel conversion, ionic liquid and the experimental design process for optimization of biodiesel production. Chapter 3 elaborates the materials and methods used in the present study which includes cultivation of microalgae, lipid extraction, transesterification process and direct transesterification. Chapter 4 discusses the data analysis and results interpretation in cultivation of *Nannochloropsis* sp. cell concentration and specific growth rate. The total lipid content, morphology of cell wall, cell disruption, biodiesel yield, fatty acid methyl esters (FAMEs) composition and dielectric properties are studied in detail in Chapter 5. The effects of various simultaneous reaction process parameters on biodiesel yield are also discussed in this chapter. Finally, Chapter 6 reveals the conclusion of the present study and suggests the recommendations for improvement in future studies.

REFERENCES

Acierno, D., Barba, A. A. and D'Amore, M. (2004). Heat Transfer Phenomena during Processing Materials with Microwave Energy. *Heat Mass Transfer*, 40, 413–420.

Alamu, O. J., Waheed, M. A. and Jekayinfa, S. O. (2007). Biodiesel Production from Nigerian Palm Kernel Oil: Effect of KOH Concentration on Yield. *J. of Energy for Sustainable Development*, 11 (3), 77-82.

Allard, B. and Templier, J. (2000) Comparison of Neutral Lipid Profile of Various Trilaminar Outer Cell Wall (TLS) Containing Microalgae with Emphasis on Algaenan Occurrence. *J. of Phytochemistry*, 54, 369–380.

Al-Widyan, M. I. and Al-Shyoukh, A. O. (2002). Experimental Evaluation of the Transesterification of Waste Palm Oil into Biodiesel. *J. of Bioresource Technology*, 85, 253–256.

Amarni, F. and Kadi, H. (2010). Kinetics Study of Microwave-Assisted Solvent Extraction of Oil from Oil Cake using Hexane: Comparison with the Conventional Extraction. *J. of Innovation Food Science Emerging Technology*, 11, 322–327.

Amaro, H. M., Catarina, A. G. and Malcata, F. X. (2011). Advances and Perspectives in using Microalgae to Produce Biodiesel. *J. of Applied Energy*, 88, 3402-3410.

Amish, P., Jaswant, V., Verma, L. and Subrahmanyam, N. (2010). A Review on FAME Production Processes. *J. of Fuel*, 89, 1–9.

Anastas, P. T. and Warner J. C. (1998). *Green Chemistry: Theory and Practice*, Oxford: Oxford University Press.

Anderson, R. A. (2005). *Algal culturing techniques*. (1st Ed.) Ed West Boothbay Harbor: Academic Press Publication.

Azocar, L., Ciudad, G., Heipieper, H. J. and Navia, R. (2010). Biotechnological Processes for Biodiesel Production using Alternative Oils. *J. of Applied Microbiology Biotechnology*, 88, 621–636.

Balasubramanian, S., Allen, J. D., Kanitkar, A. and Boldor, D. (2011). Oil Extraction from *Scenedesmus Obliquus* using a Continuous Microwave System: Design, Optimization and Quality Characterization. *J. of Bioresource Technology*, 102, 3396-3409.

Balat, M. and Balat, H. (2010). Progress in Biodiesel Processing. *J. of Applied Energy*, 87(6), 1815-1835.

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. *J. of Analyst*, 120, 1897–1904.

Becker, E. W. (1994). Microalgae Biotechnology & Microbiology. Cambridge University, Cambridge.

Belarbi, E. H., Molina, E. and Chisti, Y. (2000). A Process for High Yield and Scaleable Recovery of High Purity Eicosapentaenoic Acid Esters from Microalgae and Fish Oil. *J. of Enzyme Microbial Technology*, 26, 516–529.

Bligh, E. G. and Dyer, W. J. (1959). A Rapid Method of Total Lipid Extraction and Purification. *Canadian J. of Physiology Pharmacy*, 37, 911–917.

Boldor, D., Kanitkar, A., Terigar, B. G., Leonardi, C., Lima, M. and Breintenbeck, G. A. (2010). Microwave Assisted Extraction of Biodiesel Feedstock from the Seeds of the Invasive Chinese Tallow Tree. *J. of Environmental Science Technology*, 44, 4019–4025.

Borthwick, K. A. J., Coakley, W. T., McDonnell, M. B., Nowotny, H., Benes E. and Groschl, M. (2005). Development of a novel compact sonicator for cell disruption. *J. of Microbiological Methods*, 60, 207-216.

Boussiba, S. (2000). Carotenogenesis in the Green Alga *Haeamatococcus pluvialis*: Cellular Physiology and Stress Response. *J. of Plant Physiology*, 108, 111-117.

Brachet, A., Christen, P. and Veuthey, J. L. (2002). Focused Microwave-Assisted Extraction of Cocaine and Benzoylecgonine from Coca Leaves. *J. of Phytochemical Analysis*, 13, 162–169.

Brennan, L. and Owende, P. (2010). Biofuels from Microalgae: A Review of Technologies for Production, Processing, and Extractions of Biofuels and Co-Products. *J. of Renewable and Sustainable Energy Reviews*, 14, 557-577.

Brown, C. S., Schuerger, A. C. and Sager, J. C. (1996). Growth and Photo Morphogenesis of Pepper Plants under Red Light-Emitting Diodes with Supplemental Blue or Farred Lighting. *Hortscience*, 120, 808–813.

Budarin, V., Ross, A. B., Biller, P., Riley, R., Clark, J. H. and Jones, J. M. (2012). Microalgae Biorefinery Concept Based on Hydrothermal Microwave Pyrolysis. *J. of Green Chemistry*, 14, 3251–3354.

Chan, C. H., Yusoff, R., Ngoh, G. C. and Kung, F. W. L. (2011). Microwave-Assisted Extractions of Active Ingredients from Plants. *J. of Chromatography A*, 1218, 6213–6225.

Chen, L., Song, D., Tian, Y., Ding, L., Yu, A. and Zhang, H. (2008). Application of on-line Microwave Sample-Preparation Techniques. *J. of Trends Analytical Chemistry*, 27, 151–159.

Chen, P., Min, M., Chen, Y., Wang, L., Li, Y., Chen, Q., Wang, C., Wan, Y., Wang, X., Cheng, Y., Deng, S., Hennesy, K., Lin, X., Liu, Y., Wang, Y., Martinez, B. and Ruan, R. (2009). Review of the Biological and Engineering Aspects of Algae to Fuels Approach. *International J. of Agricultural and Biological Engineering*, 2, 1–30.

Chen, W., Sommerfeld, M. and Hu, Q. (2011). Microwave-Assisted Nile Red Method for in Vivo Quantification of Neutral Lipids in Microalgae. *J. of Bioresource Technology*, 102, 135-141.

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

Cheng, Y., Lu, Y., Gao, C. and Wu, Q. (2009). Alga-Based Biodiesel Production and Optimization using Sugar Cane as the Feedstock. *J. of Energy Fuels*, 23, 4166–4173.

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. J. of Bioresource Technology*, 139, 292–299.

Chin, C., Shen, T., Su, H. and Ding, S. (2006). *Schizochytrium limacinum* SR21 as a Source of Docosahexaenoic Acid: Optimal Growth and Use as a Dietary Supplement for Laying Hens. *Australian J. of Agricultural Research*, 57, 13-20.

Chiu, S. Y., Kao, C. Y., Chen, C. H., Kuan, T. C., Ong, S. C. and Lin, C. S. (2009). Reduction of CO_2 by a High-Density Culture of *Chlorella* sp. in a Semicontinuous Photobioreactor. *J. of Bioresource Technology*, 99, 3389–3396.

Chisti, Y. (2007). Biodiesel from Microalgae. *J. of Biotechnology Advances*, 25, 294–306.

Chisti, Y. (2008). Biodiesel from Microalgae Beats Bioethanol. *J. of Trends Bioethanol*, 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. *J. of Food Process Preservative*, 30, 407-419.

Chun-Zhao, L., Feng, W., Stiles, A. R. and Chen, G. (2012). Ionic liquids for Biofuel Production: Opportunities and Challenges. *J. of Applied Energy,* 92, 406-414.

Clark, J. H. and Tavener, S. J. (2007). Alternative Solvents: Shades of Green, *J. of Organic Process and Research Development,* 11, 149-155.

Cooksey, K. E., Guckert, J. B., William, S. and Callis, P. R. (1987). Fluorometric Determination of the Neutral Lipid Content of Microalgae Cells using Nile Red. *J. of Microbiological Methods*, 6, 333-345.

Cooney, M. J., Young, G. and Nagle, N. (2009). Extraction of Bio-Oils from Microalgae: A Review. *J. of Separation Purification Review*, 38, 291-325.

Cui, Y., Rashida, N., Huc, N., Rehman, M. S. U. and Han, J. I. (2014). Electricity Generation and Microalgae Cultivation in Microbial Fuel Cell using Microalgae-Enriched Anode and Bio-Cathode. *J. of Energy Conversion Management*, 79, 674–680.

Damiani, C., Popovich, C., Constenla, D. and Leonardi, P. (2010). Lipid Analysis in *Haematococcus pluvialis* to assess its Potential Use as a Biodiesel Feedstock. *J. of Bioresource Technolology,* 101, 3801–3807.

Dawen, K. and Somenath, M. (2003). *Extraction of Semivolatile Organic Compounds from Solid Matrices: Sample Preparation Techniques in Analytical Chemistry*. Newark, N. J.: Wiley and Sons, Inc.

Demirbas, A. (2009). Progress and Recent Trends in Biodiesel Fuels. *J. of Energy Conversion and Management*. 50, 14–34.

Dote, Y., Sawayama, S., Inoue, S., Minowa, T. and Yokohama, S. (1994). Recovery of Liquid Fuel from Hydrocarbon Rich Microalgae by Thermochemical Liquefaction. *J. of Fuel*, 73, 1855-1857.

Dufreche, S., Hernandez, R., French, T., Sparks, D., Zappi, M. and Alley, E. (2007). Extraction of Lipids from Municipal Waste Water Plant Micro Organisms for Production of Biodiesel. *J. of American Oil Chemists' Society*, 84(2), 181–187.

Eevera, T., Rajendran, K. and Saradha, S. (2009). Biodiesel Production Process Optimization and Characterization to Assess the Suitability of the Product for Varied Environmental Conditions. *J. of Renewable Energy*, 34, 762–765.

Ehimen, E. A., Sun, Z. F. and Carrington, C. G. (2010). Variables Affecting the In Situ Transesterification of Microalgae Lipids. *J. of Fuel*, 89, 677–684.

Elsey, D., Jameson, D., Raleigh, B. and Cooney, M. C. (2007). Flourescent Measurement of Microalgal Neutral Lipids. *J. of Microbiological Methods*, 68, 639-642.

Erikson, N. (2008). The Technology of Microalgal Culturing. *Biotechnology Letters*, 30(9), 1525–1536.

Eskilsson, C.S. and Bjorklund, E. (2000). Analytical-Scale Microwave-Assisted Extraction. *J. of Chromatography A*, 902, 227–250.

Fisher, R. A. (1966). *The Design of Experiments*, 2nd Ed. Hafner Publishing Company, New York.

Folch, J., Lees, M. and Sloane, G.H. (1957). A Simple Method for the Isolation and Purification of Total Lipids from Animal Tissues. *J. of Biological Chemistry*, 226, 497–509.

Freedman, B., Butterfield, R. and Pryde, E. (1986). Transesterification kinetics of soybean oil. *J. of American Oil Chemical Society*, 63, 1375–1380.

Fukuda, H., Kondo, A. and Nodac, H. (2001). Biodiesel Fuel Production by Transesterification of Oils. *J of Bioscience and Bioengineering*, 92 (5), 405.

Gelin, F., Boogers, I., Noordeloos, A., Damste, J., Riegman, R., and De Leeuw, J. (1997). Resistant Biomacromolecules in Marine Microalgae of the Classes Eustigmatophyceae and Chlorophyceae: Geochemical Implications. *J. of Organic Geochemistry*, 26, 659–675.

Gerpen, J. V. (2005). Biodiesel Processing and Production. *J. of Fuel Processing Technology*, 86, 1097–1107.

Giuliano, D., Bruno, F., Antonio, A. V. and Jose, A. T. (2010). Third Generation Biofuel from Microalgae. *Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology*, 7, 1355-1366.

Gouveia, L., Oliveira, A. C. (2009). Microalgae as a Raw Material for Biofuels Production. *J. of Industrial Microbiology and Biotechnology*, 36, 269–274.

Gotelli, N. J. (1995). *A Primer of Ecology*. Sinauer, Sunderland, Massachusetts.

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. *J. of American Oil Chemistry Society*, 7, 703–726.

Grima, E. M, Belarbi, E. H., Fernandez, F. G., Medina, A. R. and Chisti, Y. (2003). Recovery of Microalgal Biomass and Metabolites: Process Options and Economics. *J. of Biotechnology Advance*, 20, 491–515.

Grobbelaar, J. U. (2004). Algal Nutrition. In Richmond, A. (Ed). *Handbook of Microalgal Culture: Biotechnology and Applied Phycology*, Blackwell, 97–115.

Guan, H. H., Feng, C., Dong, W., Xue, Wu. Z. and Gu, C. (2010). Biodiesel Production by Microalgal Biotechnology. *J. of Applied Energy*, 87, 38-46.

Guil-Guerrero, J. L., Belarbi, E. I. H. and Rebolloso-Fuentes, M. M. (2000). Eicosapentaenoic and Arachidonic Acids Purification from the Red Microalga *Porphyridium Cruentum*. *J. of Bioseparation*, 9, 299–306.

Guillard, R. R. L. (1973). Division rates. In: Stein, J. R., ed. *Handbook of Phycological Methods: Culture Methods and Growth Measurements*. Cambridge University Press, Cambridge.

Haas, M. J. and Wagner, K. (2011). Simplifying Biodiesel Production: The Direct or In-Situ Transesterification of Algal Biomass. European *J. of Lipid Science Technology*, 113, 1219–1229.

Halim, R., Brendan, G., Danquah, M. K. and Paul, A. W. (2011). Oil Extraction from Microalgae for Biodiesel Production. *J. of Bioresource Technology*, 102, 178–185.

Halim, R., Brendan, G., Danquah, M. K. and Paul, A. W. (2012). Microalgal Cell Disruption for Biofuel Development. *J. of Applied Energy*, 91 (1), 116 – 121.

Harun, R., Singh, M., Forde, G. M. and Danquah, M. K. (2010). Bioprocess Engineering of Microalgae to Produce a Variety of Consumer Products. *J. of Renewable Sustainable Energy Review*, 14, 1037–1047.

Harvey, A., Lee, J. and Zakaria, R. (2008). Reactive Extraction of Biodiesel from Rapeseed. In: Engineers AIoC, Editors. AIChE Annual Meeting. Philadelphia, United State.

Hemwimon, S., Pavasant, P. and Shotipruk, A. (2007). Microwave-Assisted Extraction of Antioxidative Anthraquinones from Roots of *Morinda citrifolia*. *J. of Separation Purification Technology*, 54, 44–50.

Horvath, I. T. and Anastas, P. T. (2007). Innovations and Green Chemistry. *J. of Chemical Review*, 107, 2169-2173.

Hossain, A. B. M. S., Salleh, A., Boyce, A. N., Chowdhury, P. and Naqiuddin, M. (2008). Biodiesel Fuel Production from Algae as Renewable Energy. *American J. of Biochemistry and Biotechnology*, 4, 250–254.

Jasvinder, S. and Sai, G. (2010). Commercialization Potential of Microalgae for Biofuels Production. *J. of Renewable and Sustainable Energy Reviews*, 14, 2596–2610.

Jeon, H. J., Lee, S. M., Park, J. S., Jung, H. H., Choi, Y. K., Lee, S. H., Yang, Y. H. and Kim, H. J. (2010). Production of Alga Biomass (*Chlorella vulgaris*) using Microbial Fuel Cell. *The 1st Asia-Oceania Innovation Summit*, 21.

John, R. D. (2009). *Extraction Techniques in Analytical Sciences: Analytical Techniques in the Series*. Kent, U. K.: John Wiley and Sons, Ltd.

Johnson, M. and Wen, Z. (2009). Production of Biodiesel Fuel from the Microalga *Chizochytrium limacinum* by Direct Transesterification of Algal Biomass. *J. of Energy fuels*, 23, 5179–5183.

Jonathan, G. H., Visser, E. A., Reichert, W. M., Heather, D. W., Grant, A. B. and Robin D. R. (2001). Characterization and Comparison of Hydrophilic and Hydrophobic Room Temperature Ionic Liquids Incorporating the Imidazolium Cation. *J. of Green Chemistry*, 3, 156–164.

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

Kappe, C. O. and Stadler, A. (2005). *Microwaves in Organic and Medicinal Chemistry*. Wiley-VCH Verlag Gmbh & Co, Weinheim.

Kaur, S., Manas, M., Srivastava, B. S., Gogoi, K. H. and Kalita, M. C. (2012). Fatty Acid Profiling and Molecular Characterization of some Freshwater Microalgae from India with Potential for Biodiesel Production. *J. of New Biotechnology*, 29, 332–344.

Khan, S., Rashmi, A., Hussain, M. Z, Prasad, S. and Banerjee, U. C. (2009). Prospects of Biodiesel Production from Microalgae in India. *J. of Renewable and Sustainable Energy Reviews*, 13(9), 2361–2372.

Kim, Y. H., Choi, Y. K., Park, J., Lee, S., Yang, Y. H., Kim, H. J., Park, T. J., Kim, Y. H. and Lee, S. H. (2012). Ionic Liquid-mediated Extraction of Lipids from Algal Biomass. *J. of Bioresource Technology*, 109, 312-315.

Kimura, K., Yamaoka, M. and Kamisaka, Y. (2004). Rapid Estimation of Lipids in Oleaginous Fungi and Yeasts using Nile Red Fluorescence. *J. of Microbiological Methods*, 56, 331-338.

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. *J. of Chromatography A*, 889, 41–49.

Koberg, M., Cohen, M., Ben-Amotz, A. and Gedanken, A. (2011). Biodiesel Production Directly from the Microalgae Biomass of *Nannochloropsis* by Microwave and Ultrasound Radiation. *J. of Bioresource Technology,* 102, 4265–4269.

Khotimchenko, S. V. and Yakovleva, I. M. (2005). Lipid Composition of the Red Alga *Tichocarpus crinitus* Exposed to Different Levels of Photon Irradiance. *J. of Phytochemistry*, 66, 73–79.

Krichnavaruk, S., Powtongsook, S. and Pavasant, P. (2007). Enhanced Productivity of *Chaetoceros calcitrans* in Airlift Photobioreactors. *J. of Bioresource Technology*, 98, 2123-2130.

Krienitz, L. and Wirth, M. (2006). The High Content of Polyunsaturated Fatty Acids in *Nannochloropsis Lmnetica* (*Eustigmatophyceae*) and its Implication for Food Web Interactions, Freshwater Aquaculture and Biotechnology. *J. of Limnologica*, 36, 204–210.

Kulkarni, M. G. and Dalai, A. K. (2006). Waste Cooking Oil-An Economic Source for Biodiesel: A Review. *J. of Industrial Engineering Chemical Research*, 45, 2901-2913.

Kusdiana, D. and Saka, S. (2004). Effect of Water Biodiesel Fuel Production by Supercritical Methanol Treatment. *J. of Bioresource Technology*, 91, 289-295.

Lam, M. K. and Lee, K.T. and Mohamed, A. R. (2010). Homogeneous, Heterogeneous and Enzymatic Catalysis for Transesterification of High Free fatty Acid Oil (Waste Cooking Oil) to Biodiesel: A Review. *J. of Biotechnology Advance*, 28, 500–518.

Lardon, L., Helias, A., Sialve, B., Steyer, J. and Bernard, O. (2009). Life-Cycle Assessment of Biodiesel Production from Microalgae. *J. of Environmental Science Technology,* 43, 6475–6481

Laura, B. and Paolo, G. (2006). *Algae: Anatomy, Biochemistry and Biotechnology*. CRC Press, Taylor and Francis Group, Broken Sound Parkway, Boca Raton, Findland.

Lateef, H., Grimes, S. and Kewcharoenwong, P. (2009). Ionic Liquids in the Selective Recovery of Fat from Composite Foodstuffs. *J. of Chemical Technology and Biotechnology*, 84, 1681-1687.

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. *J. of Bioresource Technology*, 101, 75–77.

Leung, D. Y. C. and Guo, Y. (2006). Transesterification of Neat and Used Frying Oil: Optimization for Biodiesel Production. *J. of Fuel Process Technology*, 87, 883–890.

Leung, D. Y. C., Wu, X. and Leung, M. K. H. (2010). A Review on Biodiesel Production using Catalyzed Transesterification. *J. of Applied Energy*, 87, 1083-1095.

Lewis, T., Nichols, P. D. and McMeekin, T. A. (2000). Evaluation of Extraction Methods for Recovery of Fatty Acids from Lipid-Producing Microheterotrophs. *J. of Microbiological Methods*, 43, 107–116.

Li, H., Pordesimo, L.O., Weiss, J. and Wilhelm, L. R. (2004). Microwave and Ultrasound Assisted Extraction of Soybean Oil. *J. of Transformation*. 47, 1187–1194.

Li, X., Xu, H. and Wu, Q. (2007). Large-Scale Biodiesel Production from Microalga *Chlorella protothecoides* through Heterotrophic Cultivation in Bioreactors. *J. of Biotechnology and Bioengineering*, 98, 764–771.

Li, Y. G., Xu, L., Huang, Y. M., Wang, F., Guo, C. and Liu, C. Z. (2011). Microalgal Biodiesel in China: Opportunities and Challenges. *J. of Applied Energy*, 88, 3432–3437.

Liam, B. and Philip, O. (2010). Biofuel from Microalgae: A Review of Technologies for Production, Processing and Extractions of Biofuels and Co-Products. *J. of Renewable and Sustainable Energy Reviews,* 14, 557-577.

Liang, Y. and Cui, Y. (2014). Direct Transesterification of Wet *Cryptococcus curvatus* cells to Biodiesel through use of Microwave Irradiation. *J. of Applied Energy*, 119, 438-444.

Liau, B. C., Shen, C. T., Liang, F. P., Hong, S. E., Hsu, S. L., Jong, T. T. and Chang, C. M. J. (2010). Supercritical Fluids Extraction and Anti-Solvent Purification of Carotenoids from Microalgae and Associated Bioactivity. *J. of Supercritical Fluids*, 55(1), 169-175.

Liau, C. Z., Wang, F., Stiles, A. R and Guo, C. (2012). Ionic liquid for biodiesel Production: Opportunities and Challenges. *J. of Applied Energy*, 92, 406-414.

Lidstrom, P., Tierney, J., Wathey, B. and Westman, J. (2001). Microwave Assisted Organic Synthesis–A Review. *J. of Tetrahedron*, 57, 9225-9283.

Lotero, E., Liu, Y., Lopez, D. E., Suwannakarn, K., Bruce, D. A., and Goodwin, J. G. (2005). Synthesis of Biodiesel via Acid Catalysis. *J. of Industrial Engineering Chemical Research*, 44, 5353-5363.

Lubian, L. M., Montero, O., Moreno-Garrido, I., Huertas, I. E., Sobrino, C., Valle, M. G. and Pares, G. (2000). *Nannochloropsis* (*Eustigmatophyceae*) as Source of Commercially Valuable Pigments. *J. of Applied Phycology*, 12, 249 – 255.

Lucchesi, M. E., Chemat, F. and Smadja, J. (2004). Solvent-Free Microwave Extraction of Essential Oil from Aromatic Herbs: Comparison with Conventional Hydrodistillation. *J. of Chromatography A*, 1043, 323–327.

Luque-Garcia, J. L. and Luque-De, C. M. D. (2003). Ultrasound: A Powerful Tool for Leaching. *Trends Analytical Chemistry*, 22, 41–47.

Ma, F., Clements, L. D. and Hanna, M. A. (1998). The Effects of Catalyst, Free Fatty Acids, and Water on Transesterification of Beef Tallow. *Transactions of American Society Agricultural Engineers*, 41, 1261–1264.

Ma, F. and Hanna, M. A. (1999). Biodiesel Production: A Review. *J. of Bioresource Technol*ogy, 70(1), 1–15.

Macias-Sanchez, M. D., Mantell, C., Rodriguez, M., Martinez De, L. O. E, Lubian, L. M. and Montero, O. (2005). Supercritical Fluid Extraction of Carotenoids and Chlorophyll a from *Nannochloropsis gaditana*. *J of Food Engineering*, 66, 245–251.

Man, K. L. and Keat, T. L. (2011). Renewable and Sustainable Bioenergies Production from Palm Oil Mill Effluent (POME): Win–Win Strategies toward

better Environmental Protection. *J. of Biotechnology Advances*, 29(1), 124-141.

Man, K. L. and Keat, T. L. (2012) Microalgae Biofuels: A Critical Review of Issues, Problems and the Way Forward. *J. of Biotechnology Advances*, 673–690.

Mandal, V., Mohan, Y. and Hemalath, S. (2007). Microwave Assisted Extraction-an Innovative and Promising Extraction Tool for Medicinal Plant Research. *J. of Pharmacognosy Reviews*, 1, 7–18.

Martinez-Guerra, E., Gude, V. G., Mondala, A., Holmes, W. and Hernandez, R. (2014). Extractive-Transesterification of Algal Lipids under Microwave Irradiation with Hexane as Solvent. *J. of Bioresource Technology*, 156, 240–247.

Marchetti, J. M., Miguel, V. U. and Errazu, A. F. (2007). Possible Methods for Biodiesel Production. *J. of Renewable and Sustainable Energy Reviews*, 11, 1300-1311.

Mercer, P. and Roberto, E. A. (2011). Developments in Oil Extraction from Microalgae. *European Journal of Lipid Science and Technology*, 113(5), 539-547.

Mark, J. A. and Patrick, J. W. (2005). RSM Simplified: Optimizing Process using Response Surface Methods for design of experiment. Productivity Press, Park Avenue South, New York.

Mata, T. M., Martins, A. A. and Caetano, N. S. (2010). Microalgae for Biodiesel Production and other Applications: A Review. *J. of Renewable and Sustainable Energy Reviews*, 14, 217-232.

Matthieu, V., Valerie, T., Christian, G., Franco, V. and Farid, C. (2008). Microwave-Integrated Extraction of Total Fats and Oils. *J. of Chromatography A*, 57, 1196-1197.

McMillan, J. R., Watson, I. A., Ali, M. and Jaafar, W. (2013). Evaluation and Comparison of Algal Cell Disruption Methods: Microwave, Waterbath, Blender, Ultrasonic and Laser Treatment. *J. of Applied Energy*, 103, 128-134.

Meher, L. C., VidyaSagar, D. and Naik, S. N. (2006). Technical Aspects of Biodiesel Production by Transesterification: A Review. *J. of Renewable Sustainable Energy Review*, 10, 248–268.

Mendes-Pinto, M. M., Raposo, M. F. J., Bowen, J., Young, A. J., and Morais, R. (2001). Evaluation of Different Cell Disruption Processes on Encysted Cells of

Haematococus pluvialis: Effects on Astaxanthin Recovery and Implications for Bio-availability. *J. of Applied Phycology*, 13, 19–24.

Meredith, R. J. (1998). Engineers' Handbook of Industrial Microwave Heating. Institution of Electrical Engineers, London.

Metaxas, A. C. (1991). Microwave Heating. *J. of Power Engineering*, 9, 237-247.

Metting, F. B. (1996). Biodiversity and Application of Microalgae. *J. of Industrial Microbiology*, 17, 477–489.

Milne, T. A., Evans, R. J. and Nagle, N. (1990). Catalytic Conversion of Microalgae and Vegetable Oils to Premium Gasoline, with Shape Selective Zeolites. *J. of Biomass*, 21, 219-232.

Minowa, T. A., Yokoyama, S. Y., Kishimoto, M. and Okakurat, T. (1995). Oil Production from Algal Cells of *Dunaliella tertiolecta* by Direct Thermochemical Liquefaction. *J. of Fuel*, 74, 1735-1738.

Miao, X. and Wu, Q. (2006). High Yield Bio-Oil Production from Fast Pyrolisis by Metabolic Controlling of *Chlorella Protothrcoides*. *J. of Biotechnology*, 110, 85-93.

Miao, X. and Wu, Q. (2006). Biodiesel Production from Heterotrophic Microalgal oil. *J. of Bioresource Technology*, 97, 841–846.

Milione, M. and Zeng, C. S. (2007). The Effects of Algal Diets on Population Growth and Egg Hatching Success of the Tropical Calanoid Copepod, *Acartia Sinjiensis*. *J. of Aquaculture*, 273, 656 – 664.

Miri, K., Moshe, C., Ami, B. A. and Aharon, G. (2011). Biodiesel Production Directly from the Microalgae Biomass of *Nannochloropsis* by Microwave and Ultrasound Radiation. *J. of Bioresource Technology*, 102, 4265–4269.

Molina, G. E. (1999). Microalgae, Mass Culture Methods. In Flickinger, M. C., Drew, S.W. (Ed). *Encyclopedia of Bioprocess Technology: Fermentation, Biocatalysis and Bioseparation*, 3, 1753-1769.

Molina, G. E., Belarbi, E. H., Acien-Fernandez, F. G., Robles, M. and Chisti, Y. (2003). Tubular Photobioreactor Design for Algal Cultures. *J. of Biotechnology*, 92(20), 113-131.

Montgomery, D. C. (2009). *Design and Analysis of Experiments*. New York: John Wiley & Sons.

Mondala, A., Liang, K., Toghiani, H., Hernandez, R. and French, T. (2009). Biodiesel Production by In-Situ Transesterification of Municipal Primary and Secondary Sludges. *J. of Bioresource Technology*, 100, 1203–1210.

Mouahid, A., Crampon, C., Toudji, S. A. A. and Badens, E. (2013). Supercritical CO_2 Extraction of Neutral Lipids from Microalgae: Experiments and Modeling. *J. of Supercritical Fluids*, 77, 7-16.

Muller, F. A., Le, G. R., Herve, A. and Durand, P. (1998). Comparison of Artificial Light Photobioreactors and other Production Systems using *Porphyridium Cruentum*. *J. of Applied Phycology*, 10(1), 83–90.

Mullin, J. (1997). Microwave Processing. In G. W. Gould. (Ed.). *New Methods of Food Preservation*. London: Chapman and Hill, 112-134.

Myers, R.H. and Montgomery, D. C. (2002). Response Surface Methodology: Process and Product Optimization using Designed Experiments, second ed. Wiley, New York.

Nagle, N. and Lemke, P. (1990). Production of Methyl Ester Fuel from Microalgae. *J. of Applied Biochemistry and Biotechnology*, 24, 355–361.

Noshadi, I., Amin, N. A. S. and Parnas, R. S. (2012). Continuous Production of Biodiesel from Waste Cooking Oil in a Reactive Distillation Column Catalyzed by Solid Heteropolyacid: Optimization using Response Surface Methodology (RSM*). J. of Fuel*, 94, 156–164.

Nuchter, M., Ondruschka, B., Bonrath, W. and Gum, A. (2004). Microwave Assisted Synthesis: A Critical Technology Overview. *J. of Green Chemistry*, 6, 128–141.

Olaizola, M. (2003). Commercial Development of Microalgal Biotechnology: from the Test Tube to the Marketplace. *Biomolecular Engineering*, 20, 459-466.

Olivier-Bourbigou, H., Magna, L., Morvan, D. (2010). Ionic Liquids and Catalysis: Recent Progress from Knowledge to Appications. *J. of Catalysis A: General*, 373(1-2), 1-56.

Owen, N. A., Inderwildi, O. R. and King, D.A. (2010). The Status of Conventional World Oil Reserves-Hype or Cause for Concern. *J. of Energy Policy*, 38, 4743–4749.

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*. *J. of Biochemical Engineering*, 12, 71–77.

Parmar, A., Singh, N. K., Pandey, A., Gnansounou, E. and Madamwar, D. (2011). Cyanobacteria and Microalgae: A Positive Prospect for Biofuels. *J. of Bioresource Technology*, 102, 10163–10172.

Patil, P. D, Gude, V. G., Mannarswamy, A., Cooke, P., Nirmalakhandan, N., Lammers, P. and Deng, S. G. (2012). Comparison of Direct Transesterification of Algal Biomass under Supercritical Methanol and Microwave Irradiation Conditions. *J. of Fuel*, 97, 822-832.

Patil, P. D., Gudea, V. G., Mannarswamy, A., Cooke, P., Munson-McGee, S., Nirmalakhandan, N. (2011). Optimization of Microwave-Assisted Transesterification of Dry Algal Biomass using Response Surface Methodology. *J. of Bioresource Technology*, 102, 1399–1405.

Perveen, Z., Ando, H., Ueno, A., Ito, Y., Yamamoto, Y., Yamada, Y., Takagi, T., Kaneko, T., Kogame, K. and Okuyama, H. (2006). Isolation and Characterization of a Novel Thraustochytrid-like Microorganism that Efficiently Produces Docosahexaenoic Acid. *Biotechnology Letters*, 28, 197-202.

Pernet, F. and Tremblay, R. (2008). Effect of Ultrasonication and Grinding on the Determination of Lipid Class Content of Microalgae Harvested on Filters. *J. of Lipids*, 38, 1191–1195.

Perreux, L. and Loupy, A. (2001). A Tentative Rationalization of Microwave Effects in Organic Synthesis According to the Reaction Medium, and Mechanistic Considerations. *J of Tetrahedron*, 57, 9199–9223.

Pinzi, S., Garcia, I. L., Lopez-Gimenez F. J., Luque de Castro, M. D., Dorado, G. and Dorado M. P. (2009). The Ideal Vegetable Oil-Based Biodiesel Composition: A Review of Social, Economic and Technical Implications. *J. of Energy Fuel*, 23, 2325-2332.

Pittman, J. K., Dean, A. P. and Osundeko, O. (2011). The potential of Sustainable Algal Biofuel Production using Wastewater Resources. *J. of Bioresource Technology*, 102, 17–25.

Poliakoff, M., Fitzpatric, M. J., Farren, T. R. and Anastas, P. T. (2002). Green Chemistry: Science and Politics of Change. *J. of Science*, *297*, 807-810.

Popoola, T. O. S. and Yangomodou, O. D. (2006). Extraction, Properties and Utilization Potentials of Cassava Seed Oil. J. of Biotechnology, 5, 38–41.

Pulz, O. (2001). Photobioreactors: Production Systems for Phototrophic Microorganisms. *Applied Microbiology and Biotechnology*, 57, 287-293.

Pulz, O. and Scheinbenbogen, K. (1998). Photobioreactors: Design and Performance with Respect to Light Energy Input. *J. of Advances in Biochemical Engineering Biotechnology*, 59, 123-152.

Pyle, D. J., Garcia, R. A. and Wen, Z. Y. (2008). Producing Docosahexaenoic Acid (DHA)-Rich Algae from Biodiesel-derived Crude Glycerol: Effects of Impurities on DHA Production and Algal Biomass Composition. *J. of Agricultural and Food Chemistry*, 56, 3933–3939.

Ranjan, A., Patil, C. and Moholkar, V. S. (2010). Mechanistic Assessment of Microalgal Lipid Extraction. *J. of Industrial and Engineering Chemistry Research*, 49, 2979–85.

Reichard, C. (2004). *Solvents and Solvent Effects in Organic Synthesis*, Germany: WILEY-VCH.

Reichardt, C. (1988). Solvents and Solvent Effects in Organic Chemistry. VCH Publishers: New York.

Richmond, A. (2003). Efficient Use of Strong Light for High Photosynthetic Productivity: Interrelationships between the Optical Path, the Population Density and the Cell Growth Inhibition. *J. of Bimolecular Engineering*, 20, 229–236.

Richmond, A. (2004). *Handbook of Microalgal Culture: Biotechnology and Applied Phycology*. Garsington Road: Blackwell Science Publication.

Richter, B. E., Jones, B. A., Ezzell, J. L., Porter, N. L., Avdalovic, N., and Pohl, C. (1996). Accelerated Solvent Extraction: A Technique for Sample Preparation. *J. of Analytical Chemistry*, 68, 1033–1039.

Rodolfi, L., Chini, Z. G., Bassi, N. and Padovani, G. (2009). Microalgae for Oil: Strain Selection. Induction of Lipid Synthesis and Outdoor Mass Cultivation in a Low-Cost Photobioreactor. *Biotechnology and Bioengineering*, 102, 1-6.

Ross, A. B., Biller, P., Kubacki, M. L., Li, H., Lea-Langton, A. and Jones, J. M. (2010). Hydrothermal Processing of Microalgae using Alkali and Organic Acids. *J. of Fuel*, 89, 2234–2243.

Routray, W. and Orsat, V. (2011). Microwave-Assisted Extraction of Flavonoids: A Review, *Food and Bioprocess Technology*, 5, 1–16.

Samori, C., Torri, C., Samori, G., Fabbri, D., Galletti, P. and Guerrini, F. (2010). Extraction of Hydrocarbons from Microalga *Botryococcus braunii* with Switchable Solvents. *J. of Bioresource Technology*, 101, 3274–3279.

Sanchez, M. A., Contreras, G. A., Garcia, C. F., Molina, G. E. and Chisti, Y. (1999). Comparative Evaluation of Compact Photobioreactors for Large-Scale Monoculture of Microalgae. *J. of Biotechnology*, 70, 249–270.

Shakinaz, A., El-Sherbiny, A., Refaat, A. and Shakinaz, T. (2010). Production of Biodiesel using the Microwave Technique. *J. of Advanced Research*. 1, 309–314.

Sharma, Y. C., Singh, B. and Upadhyay, S. N. (2008). Advancements in development and characterization of biodiesel: A Review. *J. of Fuel*, 87, 2355-2373.

Shen, Y., Zhijian, P., Wenqiao, Y. and Enrong, M. (2010). Effect of Nitrogen and Extraction Method on Algae Lipid Yield. *International Journal of Agriculture and Biological Engineering*, 2(1), 51-56

Shiu, P. J., Setiyo, G., Wen-Hao, H., Novy, S. K. and Yi-Hsu, J. (2010). Biodiesel Production from Rice Bran by a Two-Step in-Situ Process. *J. of Bioresource Technology*, 101, 984-989.

Sobczuk, T., Camacho, F., Grima, E. and Chisti, Y. (2006). Effects of Agitation on the Microalgae *Phaeodactylum tricornutum* and *Porphyridium cruentum*. *J. of Bioprocess and Biosystems Engineering*, 28, 243-250.

Soh, L. and Zimmerman, J. (2011). Biodiesel Production: The Potential of Algal Lipids Extracted with Supercritical Carbon Dioxide. *J. of Green Chemistry*, 13, 142–149.

Spigno, G. and De Faveri, D. M. (2009). Microwave-Assisted Extraction of Tea Phenols: A Phenomenological Study. *J. of Food Engineering*, 93, 210-217.

Spolaore, P., Joannis-Cassan, C., Duran, E., Isambert, A., (2006). Commercial Applications of Microalgae. *J. of Bioscience and Bioengineering*, 101, 87– 96.

Suchar, V. A. and Chigbu, P. (2006). The Effects of Algae Species and Densities on the Population Growth of the Marine Rotifer, *Colurella Dicentra*. *J. of Experimental Marine Biology Ecology*, 337, 96 – 102.

Suhm, J. (2000). Rapid Wave Microwave Technology for Drying Sensitive Products. *The American Ceramic Society Bulletin*, 4, 69-71.

Terigar, B. G., Balasubramaniam, S. and Boldor, D. (2010). An Analysis of the Microwave Dielectric Properties of Solvent-Oil Feedstock Mixture at 300-3000 MHz. *J. of Bioresource Technology*, 101, 6510-6515.

Terry, K. L. and Raymond, L. P. (1985). System Design for the Autotrophic Production of Microalgae. *J. of Enzyme Microbial Technology*, 7, 474-487.

Thi, T. Y. D., Balasubramaniam, S. and Jeffrey, P. O. (2011). Screening of Marine Microalgae for Biodiesel Feedstock. *J. of Biomass and Bioenergy*, 37(7), 2534-2544.

Tierney, J. and Lidstrom, P. (2005). *Microwave-Assisted Organic Synthesis*. Oxford: Blackwell Scientific.

Torzillo, G., Carlozzi, P., Pushparaj, B., Montaini, E. and Materassi, R. (1993). A Two-Plane Tubular Photobioreactor for Outdoor Culture of *Spirulina*. *J. of Biotechnology and Bioengineering*, 42, 891-898.

Tredici, M. R. (1999). Bioreactors, Photo. In Flickinger, M. C. and Drew, S. W, (Ed). *Encyclopedia of Bioprocess Technology: Fermentation, Biocatalysis and Bioseparation*, 395-419, Wiley.

Tsigie, Y. A., Huynh, L. H., Ismadji, S., Engida, A. M. and Ju, Y. (2012). In situ Biodiesel Production from Wet *Chlorella vulgaris* under Subcritical Condition. *J. of Chemical Engineering*, 213, 104–108.

Uduman, N., Qi, Y., Danquah, M. K., Forde, G. M. and Hoadley, A. (2010). Dewatering of Microalgal Cultures: A Major Bottleneck to Algae-based Fuels. *J. of Renewable and Sustainable Energy*, 2, 1-15.

Ugwu, C. U., Ogbonna J., Tanaka, H. (2002). Improvement of Mass Transfer Characteristics and Productivities of Inclined Tubular Photobioreactors by Installation of Internal Static Mixers. *J. of Applied Microbiology and Biotechnology*, 58(5), 600-607.

Um, B. H., Kim, Y.S., (2009). Review: A Chance for Korea to Advance Algal-Biodiesel Technology. *J. of Industrial Engineering Chemistry*, 15, 1–7.

Vicente, G., Bautista, L. F., Rodriguez, R., Gutierrez, F. J., Sadaba, I., Ruiz-Vazquez, R. M., Torres-Martinez, S., and Garre, V. (2009). Biodiesel Production from Biomass of an Oleaginous Fungus. *J. of Biochemical Engineering*, 48, 22-27.

Vijayaraghavan, K. and Hemanathan, K. (2009). Biodiesel Production from Freshwater Algae. *J. of Energy Fuels*, 23, 5448–5453.

Vynas, A. P., Verma, J. L. and Subrahmanyam, N. (2010). A Review on FAME Production Processes. *J. of Fuel*, 89, 1-9.

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 Mixed Cultures. *J. of Bioresource Technology*, 102, 2724–2730.

Warabi, Y., Kusdiana, D. and Saka, S. (2004). Reactivity of Triglycerides and Fatty Acids of Rapeseed Oil in Supercritical Alcohols. *J. of Bioresource Technology,* 91, 283–287.

Watanabe, Y. and Saiki, H. (1997). Development of a Photobioreactor incorporating *Chlorella* sp. for removal of CO_2 in Stack Gas. *J. of Energy Conversion and Management,* 38, 499-503.

Wee, F. H., Soh, P. J., Suhaizal, A. H. M., Nornikman, H. and Ezanuddin, A. A. M. (2009). Free Space Measurement Technique on Dielectric Properties of Agricultural Residues at Microwave Frequencies. *International Microwave and Optoelectronics Conference (IMOC 2009)*, Belen, 178-182.

Welton, T. (2004). Ionic Liquids in Catalysis. *J. of Chemical Review*, 248, 2459–2477.

Widjaja, A., Chien, C. C. and Ju, Y. H. (2009). Study of increasing Lipid Production from Fresh Water Microalgae *Chlorella vulgaris*. *J. of Taiwan Institute of Chemical Engineering*, 40, 13–20.

Wiltshire, K. H., Boersma, M., Moller, A. and Buhtz, H. (2009). Extraction of Pigments and Fatty Acids from the Green Alga *Scenedesmus obliquus (Chlorophyceae)*. *J. of Aquatic Ecology*, 34, 119–126.

Wu, S., Yu, S. and Lin, L. (2005). Effect of Culture Conditions on Docosahexaenoic Acid Production by *Schizochytrium* sp. S31. *J. of Process Biochemistry*, 40, 3105-3108.

Xiong, W., Li, X. F., and Wu, Q. Y. (2000). High-Density Fermentation of Microalga *Chlorella protothecoides* in Bioreactor for Microbio-Diesel Production. *J. of Applied Microbiology Biotechnology,* 27, 312-318.

Xu, H., Miao, X. and Wu, Q. (2006). High quality biodiesel production from a microalga *Chlorella protothecoides* by heterotrophic growth in fermenters. *J. of Biotechnology*, 126(4), 499–507.

Xue, L.,Brilman, D. W. F., Withag, J. A. M., Brem, G. and Kersten, S. (2011). Assessment of a dry and a wet Route for the Production of Biofuels from Microalgae: Energy Balance Analysis. *J. of Bioresource Technology*, 102, 5113–5122.

Yao, L., Gerde, J. and Wang, T. (2012). Oil Extraction from Microalga *Nannochloropsis* sp. with Isopropyl Alcohol. *J. of American Oil Chemists' Society*, 89, 2279–2287.

Yoo, C., Jun, S. Y., Lee, J. Y., Ahn, C. Y. and Oh, H. M. (2010). Selection of Microalgae for Lipid Production under High Levels Carbon Dioxide. *J. of Bioresource Technology*, 101, 71-74.

Young, G., Nippgen, F., Titterbrandt, S. and Cooney, M. J. (2010). Lipid Extraction from Biomass using Co-Solvent Mixtures of Ionic Liquids and Polar Covalent Molecules. *J. of Separation and Purification Technology*, 72, 118-121.

Zhang, Y., Dude, M. A., McLean, D. D. and Kates, M. (2003). Biodiesel Production from Waste Cooking Oil: Process Design and Technological Assessment. *J. of Bioresource Technology*, 89, 1-6.

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. *J. of Bioresource Technology*, 101, 931-936.

Zheng, H., Wang, Y. Z., Kong, J. H., Nie, C. and Yuan, Y. (2010). Ionic Liquid-based Microwave Assisted Extraction of Rutin from Chinese Medical Plants. *J. of Talanta*, 83, 582-590.

Zhi-Yuan, L., Guang-Ce, W. and Bai-Cheng, Z. (2008). Effect of Iron on Growth and Lipid Accumulation in *Chlorella vulgaris*. *J. of Bioresource Technology*, 99, 4717-4722.