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A STUDY ON THE ULTRASONIC OIL EXTRACTION AND *IN SITU* TRANSESTRIFICATION OF MICROALGAE BIODIESEL

BUDI WIYARNO

A thesis submitted in fulfillment of the requirements
for the award of the degree of
Master Engineering in Chemical Engineering

Faculty of Chemical and Natural Resources Engineering
UNIVERSITI MALAYSIA PAHANG

SEPTEMBER 2012

SUPERVISOR'S DECLARATION

I hereby declare that I have checked this thesis and in my opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Master of Engineering in Chemical Engineering

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STUDENT'S DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged. The thesis has not been accepted for any degree and is not concurrently submitted for award of other degree.

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DEDICATION

In the name of Allah, The Most Gracious and The Most Merciful

I humbly dedicate this thesis to…

my beloved wife; Lusi Nurhayati,

my lovely son and daughter; Ukasyah Najmulhaq Wiyarno and Asha Aruna Wiyarno

my beloved parents; Ibu, Bapak (alm), Mamah, and Abah

my great family members,

my best friends,

those who has influenced my life and each me to walk on the right path

thank you…

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Praise be to ALLAH SWT who has given me the blessings so that I could finish my thesis.

I would like to take this precious moment to express my sincere appreciation and wholehearted appreciation to my supervisors, Prof. Dato` DR Rosli Mohd Yunus, and Assoc Prof. Dr Maizirwan Mel for their consistent encouragement, keen effort in respect to technical assistance and continuous guidance throughout the course of this study.

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I would also like to address my unlimited thanks to my wife; LusiNurhayati, my son and daughter: Ukasyah Najmulhaq Wiyarno and Asha Aruna Wiyarno, Ibu, (alm) Bapak Pekalongan, Mamah and Bapak Sumedang, my sisters and brothers for their patience, love, trust and bottomless support.

Finally, I realize that this thesis is still far from being perfect. However, I hope that this will be of some contribution to the development of new energy for the world.

ABSTRACT

Ottawa-Gatineu, Predicted that in 2020 Oil and LNG production both types of fossil fuels is declining. These conditions encourage countries in the world to perform efficiency and explore the potential of new energy source and perform diversification of fuel oil. One of the alternative energy sources that can be proposed is biofuel

Biofuel to have become the focus of many researchers in many countries There are many of the biodiesel sources which are derived from the seeds of the plants, but at the same time people need these plants for food supply (Palm oil, coconut oil, Soybean oil, corn oil, etc). The use of microalgae to replace fossil fuel has become one focus of attention as the use of this plant is beneficial since it has lesser or nearly no effect to the world's food supply

The extraction and transestrification of microalgae oil are interesting topics (besides culturing and microalgae strain) in the development process of biodiesel microalgae. Some methods of biodiesel production have been well known. In this research, the ultrasonic method was used to identify the dominant factors in the extraction and *in situ* transesterification processes and to determine the optimum combination of the dominant factors. This is an experimental laboratory study that was run using ultrasonic homogenizer Omni Ruptor 4000, examining the effect of type of solvent, solvent concentration, alga-solvent ratio, ultrasonic power, ultrasonic time, ultrasonic pulse and mixing toward yield. Based on Box-Behnken design, a quadratic model is developed to correlate the parameter to surface area to analyze certain factors and combination of dominant factors.

The result shows that power, time and pulse as the most dominant factors that influence the yield. In the extraction, the combinations of pulse-time give better result than power-pulse combination. While in the *in situ* transesterification, the power-time combination give better result that power-pulse combination. Even though the optimum point has not been reached yet, in general the combination of

power-time is categorized as the most influential combination to increase the yield.

The experimental values versus predicted values use the model equation developed by STATISTICA Software version 6.0. A line of unit slope, the line of perfect fit with points corresponding to zero error between experimental and predicted values is also shown that the coefficient of correlation (R^2) is 0.97977 (for extraction) and 0.98743 (for *in-situ*). Saponication number is 114, 269 KOH/1 g oil. The percentage of FFA is 19.67% consisting of monounsaturated and polyunsaturated Octadecenoic acid (C18:1) 43.49%, Dedecanoic acid (C12) 16.30%, Hexadecanic acid (C16:0) 12.51%, Tetradecanoic acid (C14) 11.43%, Octadecadinoic acid (C18:2) 5.85% dan Octadecanoic acid (C18:0) 5.62%.

ABSTRAK

Ottawa-Gatineu telah membuat jangkaan bahawa pada tahun 2020 pengeluaran dua jenis bahan api fosil iaitu minyak dan LNG akan semakin merosot. Hal ini menyebabkan beberapa negara di dunia berusaha dengan lebih efisyen untuk meneroka sumber tenaga baru dan mempelbagaikan bahan api. Salah satu sumber tenaga alternatif yang boleh dicadangkan ialah biofuel.

Biofuel telah menjadi tumpuan ramai penyelidik di banyak negara. Terdapat banyak sumber biodiesel yang berasal dari benih tumbuh-tumbuhan, tetapi pada masa yang sama tumbuh-tumbuhan diperlukan untuk bekalan makanan (minyak kelapa sawit, minyak kelapa, minyak kacang soya, minyak jagung, dll). Penggunaan mikroalga untuk menggantikan bahan api fosil telah menjadi salah satu fokus perhatian kerana penggunaan tumbuhan ini mempunyai kurang atau hampir tiada kesan kepada bekalan makanan dunia.

Perahan dan transestrifikasi minyak Mikroalga adalah topik yang menarik – selain pengkulturan dan jenis Mikroalga - dalam proses pembangunan biodiesel Mikroalga. Beberapa kaedah pengeluaran biodiesel telah pun terkenal. Dalam kajian ini, kaedah ultrasonic telah digunakan bagi mengenalpasti faktor-faktor dominan dalam proses perahan dan transesterifikasi *in situ* dan menentukan keadaan optimum bagi kombinasi tertentu dari faktor yang dominan. Ini adalah satu kajian makmal uji kaji yang dijalankan menggunakan homogenizer ultrasonik Omni Ruptor 4000, dengan memeriksa kesan jenis pelarut, kepekatan pelarut, nisbah alga-pelarut, kuasa ultrasonik, masa ultrasonik, denyutan ultrasonic dan campuran terhadap hasil. Berdasarkan rekabentuk Box-Behnken, model kuadratik dibangunkan untuk mengaitkan parameter dengan luas permukaan untuk menganalisis faktor-faktor tertentu dan gabungan faktor-faktor dominan.

Hasilnya menunjukkan bahawa kuasa, masa dan denyutan sebagai faktor-faktor yang paling dominan yang mempengaruhi hasil. Dalam perahan, gabungan masa-denyutan memberikan hasil yang lebih baik dari gabungan kuasa-denyutan. Ketika dalam transesterifikasi *in situ,* gabungan kuasa-masa memberi keputusan yang lebih baik dari gabungan kuasa-denyutan. Walaupun titik optimum masih

belum tercapai, secara umumnya gabungan kuasa-masa dikategorikan sebagai gabungan yang paling berpengaruh untuk meningkatkan hasil.

Nilai eksperimen berbanding nilai ramalan menggunakan persamaan model yang dibangunkan oleh Perisian Statistica versi 6.0. Garis cerun unit, garis patut sempurna dengan titik-titik yang sepadan dengan ralat sifar di antara nilai-nilai uji kaji dan nilai ramalan juga menunjukkan bahawa pekali korelasi (R2) adalah 0.97977 (untuk perahan) dan 0.98743 (untuk *in situ*). Nombor saponication adalah 114,269 KOH/1g minyak. Peratusan FFA adalah 19.67% yang terdiri dari asid Octadecenoic (C18: 1) mono tidak tepu dan poli tidak tepu sebanyak 43.49%, asid Dedecanoic (C12) sebanyak 16.30%, asid Hexadecanic (C16: 0) sebanyak 12.51%, asid Tetradecanoic (C14) sebanyak 11.43%, asid Octadecadinoic (C18: 2) sebanyak 5.85% dan asid Octadecanoic (C18: 0) sejumlah 5.62%.

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c	Concentration
C	Sound velocity
C_A	Concentration of A
c_{L1}	Bulk fluid concentration
c_{Li}	Concentration in the fluid next to the surface of the solid
c_p	Specific heat at constant pressure
c_t	Concentration at time t
c_∞	Concentration at equilibrium
c_v	Specific heat
D	Diffusion
D_{AB}	Molecular diffusivity of the molecule A and B
D_{ac}	Diffusion coeffiecient in the sound field
E	Bulk modulus of elasticity
E_k	Kinetik energy
E_p	Potential energy
I_o	Intensity of the sound wave
J_{*AZ}	Molar flux of component A in the z direction
k_c	Mass transfer coefficient
K_o	Extraction rate constant
N_A	Rate of convective mass transfer
p	Sound pressure
P_a	Acoustic pressure
P_h	Normal atmospheric pressure
P_L	Total liquid pressure
T	Temperature
u	Velocity of displacement
v	Viscosity
V	Acoustic particle velocity amplitude

V_o	Steady state volume
x_A	Mole fraction of A
x	Path traversed by the sound wave
z	Distance of diffusion
ΔT	Temperature difference
β^*	Thermal expansion coefficient
ε_M	Turbulent or mass eddy diffusivity
η	Dynamic viscosity coefficient
ρ	Density of the medium
ω	Cyclic frequency of the sound wave
χ	Second viscosity coefficient

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CHAPTER 1

INTRODUCTION

1.1 Research Background

So far, fossil fuel remains a major source of world energy to sustain almost all sectors of human activity. From all sectors, industry and transport sector uses 60% of total energy needs, followed by domestic sector 22% and 18% commercial (Ian Charles, 2005). The need of fuel keeps increasing as world population growth; however it is not balanced with the production of world's oil and liquid gas. It is predicted that in 2020 the production of both types of fossil fuels is declining (Ottawa-Gatineu, 2008). These conditions encourage countries in the world to perform efficiency and explore the potential of new energy source and perform diversification of fuel oil.

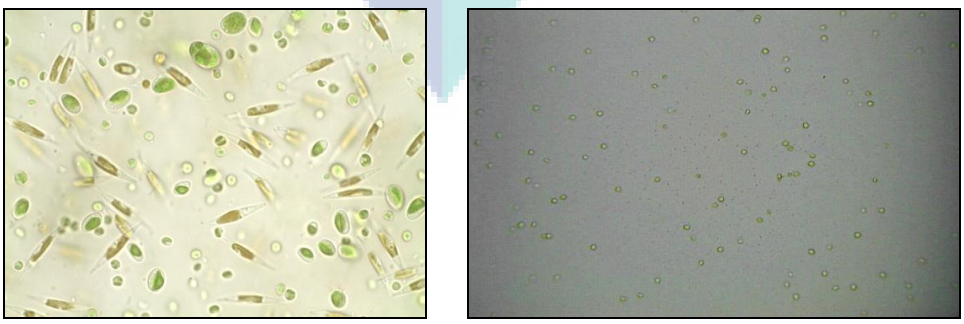
In attempt to solve the problem above, exploration of new energy sources needs to be done. One of the alternative energy sources that can be proposed is biofuel. Biofuel can be categorized as a renewable energy. Although the scale is relatively small in number, nowadays approximately 13% of world's energy is renewable. The U.S.A has substituted 6% of the total energy need to be a renewable energy, starting from solar energy, vegetable (biofuel), waste, wind and others (Ian Charles, 2005).

Biofuels, like biodiesel, play a very important role as alternative energy and green energy. It can replace petroleum fuel nowadays. It can be produced from organic material, such as plants, and can be renewed. High quality biofuel, in general, can be obtained from the feedstock, not the food stock. This can reduce 50% emission of greenhouse effect comparing to fossil fuel. Microalgae, as one source for biofuel, it is an example of potential renewable energy source.

The idea to use microalgae as fuel is not a new one. In 1970's, America's NREL (National Renewable Energy Laboratory) conducted some studies on its screening, genetic engineering and mass production (Feinberg, 1978). Moreover in 1957, Golueke processed microalgae into fuel, *i.e*, methane gas, using anaerobic process.

Microalgae are plants which have one or more cells; they have chlorophyll and live in colony. They use photosynthesis process to turn sun light, carbon dioxide as well as some nutrition from water into lipid, carbohydrate and protein and release the oxygen. Based on their habitat, algae are divided into two: fresh water and sea water (Richmond, 2004). Some microalgae are potential source of biodiesel fuel because their lipid level is high (Li, 2008; Chisti, 2007).

Daniel Feinberg (1978) investigated the composition of 11 kinds of microalgae and he found that 4 species are identified to have high lipid component, 3 species are identified to have high hydrocarbon, and 3 species contain high protein and 1 species produce high glycerol. *Nannochloropsis* sp (Figure 1.1) is a kind of microalgae which is potential for basic material of biofuel (Feinberg, 1978; Mata, 2009). Some studies report that its lipid level is 54 % (Hill and Feinberg, 1984) and 12-53% (Mata, 2009). The lipid productivity level of *Nannochloropsis* sp is 27.00 $mg.L^{-1}.day^{-1}$ (Brennan, 2009), and 37.6-90.0 $mg.L^{-1}.day^{-1}$ (Mata, 2009).



(A) (B)

Figure 1.1 *Nannochloropsis Sp*. (A) *Occulata Sp*. (B)

Extraction is one method used to obtain oil from plant. Some of the well-known extraction models are pressure, solvent/soxhlet, osmosis pressure, microwave, supercritical and ultrasonication (Shweta, 2005; Szentmihalyi *et al*, 2002). Ultrasonic become one of the methods began to be applied in the process of oil extraction plant. There are two reasons why Ultrasonic is chosen in this study. First, it has low operational temperature, and second it has a relatively short operational time (Shweta, 2005). It is known that high temperature will comprise more but low quality oil (Liauw, 2008) and conventional extraction takes a longer time (Dong, 2004). Hitherto, the use of ultrasonic to assist extraction has been done to some seeds such as seeds of tobacco, fennel, peganum, seed, rose hip, sunflower, soybean, and rape (Ivana, 2007). Microalgae powder of *Nannochloropsis sp.* is used in this study.

Cavities resulted by ultrasonic sound trigger crash or collision among particles in the body of the cell which furthermore increase the heat. This heat, make breaks the cell, and finally releases the oil from the cell. This extraction process is faster comparing with the traditional methods, because the contact surface area between solid and liquid phase is much greater, due to particle disruption taking place (Palma, 2005). Ethanol (Liauw, 2008; Zhen, 2008) is normally used to extract microalgae via assisted ultrasonic energy. This research uses soxhlet extraction method as comparison to ultrasound extraction method.

1.2 Problem Statement

There are two influential steps to get the best result in the microalgae-based biodiesel processing. The first has to do with the type of strain used and also the environment. The second is the oil extraction process from the microalgae cell. Some extraction methods to get the oil from microalgae have been known, but nowadays the best method is still under investigation.

Microalgae have only one cell. They have some cell walls that are very difficult to penetrate by the solvent, so that it needs efforts to break the cell wall to ease the solvent penetration. Large solvent penetration will increase the oil yield. This oil will furthermore be converted into biodiesel.

The biodiesel processing in general involves two steps, namely extraction and transesterifiction. The material which has high fatty acid even needs more than two steps. This condition causes inefficiency of catalyst and reaction time. For this reason, a treatment focusing on breaking the cell walls and speeding the reaction time is needed.

Ultrasonication is another potentially useful disruption method. Ultrasound is high acoustic intensities is known to disrupt microbial cells in suspension (Medina, 1998). Ultrasonic Assisted in the extraction process and *in situ* transesterification potential to reduce the reaction time, reduce the static separation time, increase of biodiesel yield, reduce the catalyst required up to 66% processing.

The extraction and *in situ* transestrification processes of microalgae using ultrasonic are influenced by many factors. From the time being, the research done focused on comparing one particular factor on the oil yield, for example ultrasonic toward yield, time toward yield, pulse toward yield, and amplitude toward yield. However, a study on the interaction between several factors to identify the best combination that provide optimum results has not been conducted.

1.3 Objective of the Research

The objectives of this research is to identify the most influential factors in the process of oil extraction and *in situ* transesterification of microalgae assisted by ultrasonic; and to analyze the interaction among factors to determine the optimum condition in the process.

1.4 Significant of the Research

Oil extraction from algae is a hot topic discussed by the researchers at this time. Extraction is a costly process for the sustainability of microalgae as the source of biodiesel.

Some extraction methods have been worked out; some of them are solvent extraction, expeller extraction, enzymatic extraction, supercritical extraction, osmotic extraction, electromechanical extraction and ultrasonic extraction.

In some extraction cases, some problems were identified, they are a long extraction time, type of solvent used, and the big amount of the solvent, the expensive cost, and unsatisfying result.

Laboratory-scale research has compared some methods of extraction of microalgae (Yon, 2009). It is known that the use of ultrasonic wave give a significant result in reducing the extraction time and the percentage of oil yielded (Zhan, 2008). This research tries to review some factors influencing the extraction and transesterification of microalgae oil; and determine combination of each factor to get the optimum result. The result of this research is very important for the development of microalgae-based biodiesel as the future fuel source.

1.5 Scope of the Research

The scopes of this research are:

1. To identifying the significant factors influencing the extraction process and *in situ* transesterification using ultrasonic in the microalgae biodiesel processing
2. To determine the optimum condition of each significant factor during the interaction in the extraction and *in situ* transesterfication processes.
3. To identify the components of microalgae oil

1.6 Structure of Thesis

Chapter 1 introduces the fossil fuels demand and stock, alternative fuel source, microalgae potential and process issues. It is followed by the objective, scopes and methodology of this thesis.

Chapter 2 reviews some relevant literature about the culturing, the opportunity of microalgae to produce fuel, biodiesel and production process, and also ultrasonic extraction theory. Here, the *in-situ* transestrification process is also discussed.

Chapter 3 discusses some extraction methods of microalgae oil using ultrasonic and *in situ* transetrification for biodiesel using ultrasonic. By using Box-Behnken Design of industrial statistics and Six Sigma of STATISTICA version 6.0, the variables influencing the extraction process were analyzed the result in the most influential variable in the ultrasonication process. This variable used to identify correlation amongst which give optimum result in the extraction process and *in situ* transestrification. Moreover, the microalgae components, microalgae oil resulted, the influence of ultrasonic wave toward the cell structure of microalgae after the treatment were analyzed.

Chapter 4 consist of several sub sections, starting with the analysis of microalgae component, discussion on the some preliminary research which compare ultrasonic extraction and soxhlet extraction. The preliminary researches help the researcher to convince himself to use ultrasonic method in this study. In the ultrasonic assisted study, the most influential variables in the extraction and *in situ* ultrasonic transetrification were studied and analyzed. Box Bhenken Design STATISTICA v 6.0 was used in this research.

Chapter 5 summaries the result obtained from previous chapter. The recommendations for future work are outlined. The recommendations are given based on assessment of significant findings, limitations, conclusion obtained and difficulties encountered in this study.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction of Microalgae

2.1.1 Definition and Type of Algae

Algae is a group of chlorophyll plants, which consists of one or many cells, live in colony, and reproduces non-sexually. Algae use photosynthetic process, a process by which they use the energy from the sun to produce their own food. They absorb CO_2 and some nutrition from water, and then change them into lipid, carbohydrate and protein as well as release O_2. Based on the size, algae can be distinguished into two categories: the complex big-sized algae, or macroalgae and the monocell small–sized algae, or microalgae.

(A) (B)

Figure 2.1 Two types of algae: Macroalgae (A), Microalgae (B)

2.1.2 Habitat and Culturing Method

Based on the habitat, microalgae are divided into two namely fresh water microalgae and sea water microalgae. Referring to this information, the culturing method of microalgae can be done both in fresh water or marine /sea water. Culturing here means particular technique to grow microalgae in a controlled environment. The controlling is done because the growth of microalgae is influenced by some environmental factors: physical or abiotic.

In general, some aspects that should be controlled during the microalgae culturing are level of acidity (pH), temperature, salinity, CO_2 supply, light intensity, and the depth. Borowistzka (1998) has summarized this as in Table 2.1.

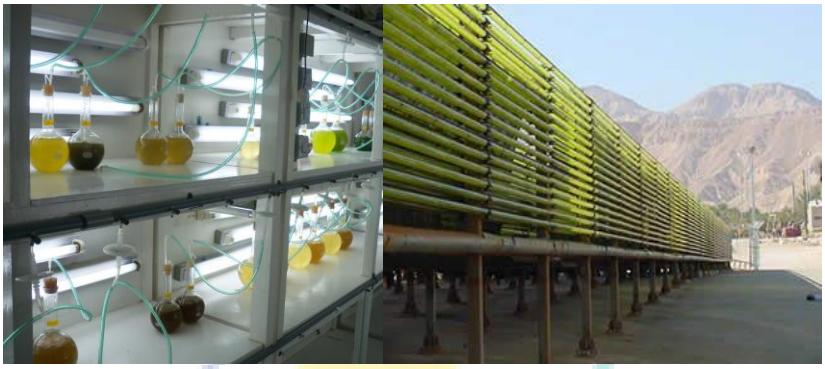
Each microalga has different specifications of media to grow, but in general microalgae can grow in a media which acidity level is pH 7-9. The temperature is approximately 20-30^oC. The temperature which is lower than 16^oC will cause the microalgae to grow slower. Meanwhile, the temperature which is more than 35^oC will cause the death for some types of microalgae. The favorable salinity was about12-40 g/l, and the light intensity was 1000 – 10.000 Cd, depending on the volume and density with photoperiodic (the comparison of light-dark) 16:8 minimum value and 24:0 maximum value. Microalgae only need 1/10 of the direct sunlight.

The addition of nutrition can increase lipid content of microalgae up to 50% and CO_2 supply in microalgae culturing will increase the productivity about 10 g C/m^2/day or increase 5 times without the aeration of CO_2.

Table 2.1. Factors influencing microalgae culturing (Borowitzka, 1998)

Factor	**Parameter**
Abiotic	Light (quality, Intensity)
	Temperature
	Nutrition concentration
	O_2
	CO_2 and pH
	Salinity
	Toxic chemical material
Biotic	Pathogen (Bacteria, fungus and Virus)
	Competition of other algae
Operation	Stirring
	Dilution
	Depth
	Harvest frequency
	Addition of bicarbonate

There are two recognized methods in microalgae culturing, the first is closed method or photobioreactor and the second is open method or open pond. The photobioreactor (Figure 2.2) is bio reactor assisted with artificial light, to replace the sunlight, to help the photosynthesis. In other words, photobioreactor is a culturing place/reactor that allows light to go through and in general it is a closed system.

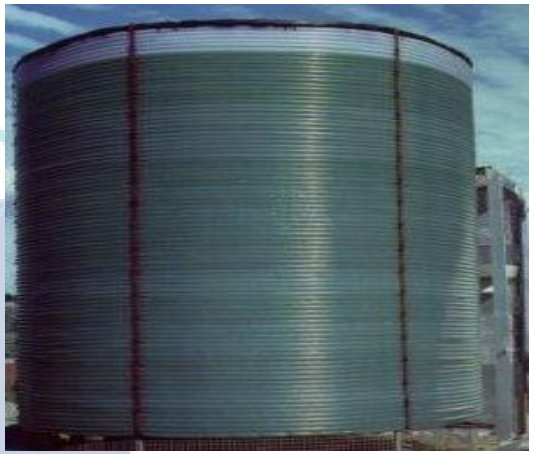


(A) (B)

Figure 2.2 Culturing with photobioreactor for the lab-scale (A) and commercial scale (B)

Microalgae culturing in the artificial ponds and container is categorized into open culturing or open pond. In general, microalgae culturing is developed shallow big ponds, tanks, circular ponds and raceway ponds (ponds that look like circuit arena). The culturing for open pond scale is now extensively done in many countries. Some of the models can be seen in Figure 2.3 below.

A. Deep Aerated Tank



B. Biocoil pond

C. Raceway pond

D. Center pivot pond

Figure 2.3 Some open pond system models

The comparison of culturing method of open pond type and photobioreactor (PBR) can be seen in Table 2.2.

Table 2.2. The comparison of culturing methods between photobioreactor and open pond (Anders, 2007)

Parameter	**Open pond**	**Photobioreactor (PBR)**
Land needed	High	Low
Water Evaporation level	High	Low
Level of CO_2 lost	High, depend on the depth	Low
O_2 concentration	Low	Low (if O_2 processing included in PBR)
Temperature	Difficult to control	Easy to control
Cleaning	No serious problem	Need attention
Contamination risk	High	Low
Microalgae quality	Not fixed	Can be maintained
Microalgae concentration	low (0.1-0.5 g/l)	High (2-8 g/l)
Flexibility of the product	Low	High
Control process	limited	Easy
Dependency on the weather	High	Medium
Start up	6-8 weeks	2-4 weeks
Capital	High (US $ 100.000/ha)	Very high (US$ 1 million/ha)
Operational cost	Low (pump, CO_2)	Very high (CO_2, pH control, O_2, cooler, cleaning etc.)
Harvest cost	high (depend on species)	Low
Commercialism	high 5000 ton/year	Low, but having additional value i.e. foods and cosmetics

2.1.3 Chemical Component and Product of Microalgae

2.1.3.1 Chemical component

Microalgae has 3 major components namely protein, carbohydrate and lipid. Lipid is grouped into 2 big components namely neutral and polar. The neutral group consists of glycerides, Wax Ester, hydrocarbon, free fatty acids and sterols. The polar lipid consists of phospholipids and glycolipids (Medina, 1998). Marine algae contain a bewildering array of major fatty acids. The major saturated fatty acid is invariably palmitate (C16:0) and, in contrast to higher plants, palmitoelate (C16:1) is the major monoene. C18 fatty acids are much less abundant than in leaves, and the C20 polyunsaturated acids are very important (Gunstone, 2007).

2.1.3.2 Food Product

So far, microalgae are used as raw material for supplement food industry and health, cosmetics and medicine. Some types of microalgae used for supplement food are Isochrysis, Chaetoceros, Chlorella, Spirulina and Dunaliella. Table 2.3 presents some types of microalgae, product and their use.

Table 2.3. Types of Microalgae, their product and their use (Anders, 2007)

Species	**Product**	**Use**	**Culturing type**	**Reference**
Spirulina	Phycocyanin Biomass	Health foods and cosmetics	Open pond	Lee (2001); Costa *et al*. (2003)
Chlorella Vulgaris	Biomass	Health foods, supplements, substitutive foods.	Open pond, Photobioreaktor	Lee (2001)
D. Salina	Caroteniods, B-caroren	Health foods, supplements	Open pond	Jin and Melis (2003), Del Campo *et al*. (2007)
Haematococcuspulvaris	Carotenoids, astaxanthin	Health foods, pharmaceutical	Open pond	Del Campo *et al*. (2007)
Odontellaaurita	Fatty acids	Pharmaceutical, cosmetics, baby foods	Open pond	Pulz and Grob (2004)
Pophyridiumcruentum	Polysakarida	pharmaceutical, cosmetics	Tubular PBR	Fuentes *et al*. (1999)
Isochrysisgalbana	Fatty Acids	Animal foods	Open pond PBR	Molina Grima (1994), Pulz dan Gross (2004)
Phaedactylumtricomutum	Lipid, Fatty acids	Nutrition, green fuel	Open pond PBR	Yongmanitchai and Ward (1991), Acien-Fernandez *et al* (2003)
Lyngbya majuscule	Immune Modulator	Pharmaceutical, nutrition		Singh *et al*(2005)
Muriellopsis sp	Carotenoid Lutein	Health foods and supplements	Open pond PBR	Blanco *et al*. (2007), Del Campo *et al*. (2007)

2.1.3.3 Fuel Product

In addition to some useful products mentioned above, microalgae are also potential to be used as green fuel. Some of the fuel products resulted from microalgae are:

2.1.3.3.a Biomethane Gas

Methane is very important in electric generator system. It functions as the fuel- burner in the gas turbine or steam boiler. Compared with other hydrocarbon fuels, the methane burning could produce less CO_2 for each heat value. Research on using microalgae as the source of biomethane has been done since 1960. The result shows biomethane gas resulted from microalgae is far more potential than other biomass resources such as woods, grasses, and city rubbish. However, the production cost is 2-10 times more expensive comparing to the methane resulted from natural gas.

2.1.3.3.b Bioethanol

Ethanol from algae is produced by changing the powder inside the algae and the cellulose in its wall cell. Algae are the second generation of bioethanol resources which are rich in carbohydrate/polysaccharide and cellulose in the cell wall. Producing bioethanol from algae is not difficult, but the big challenge is in separating the component which has higher economic value than the ethanol produced.

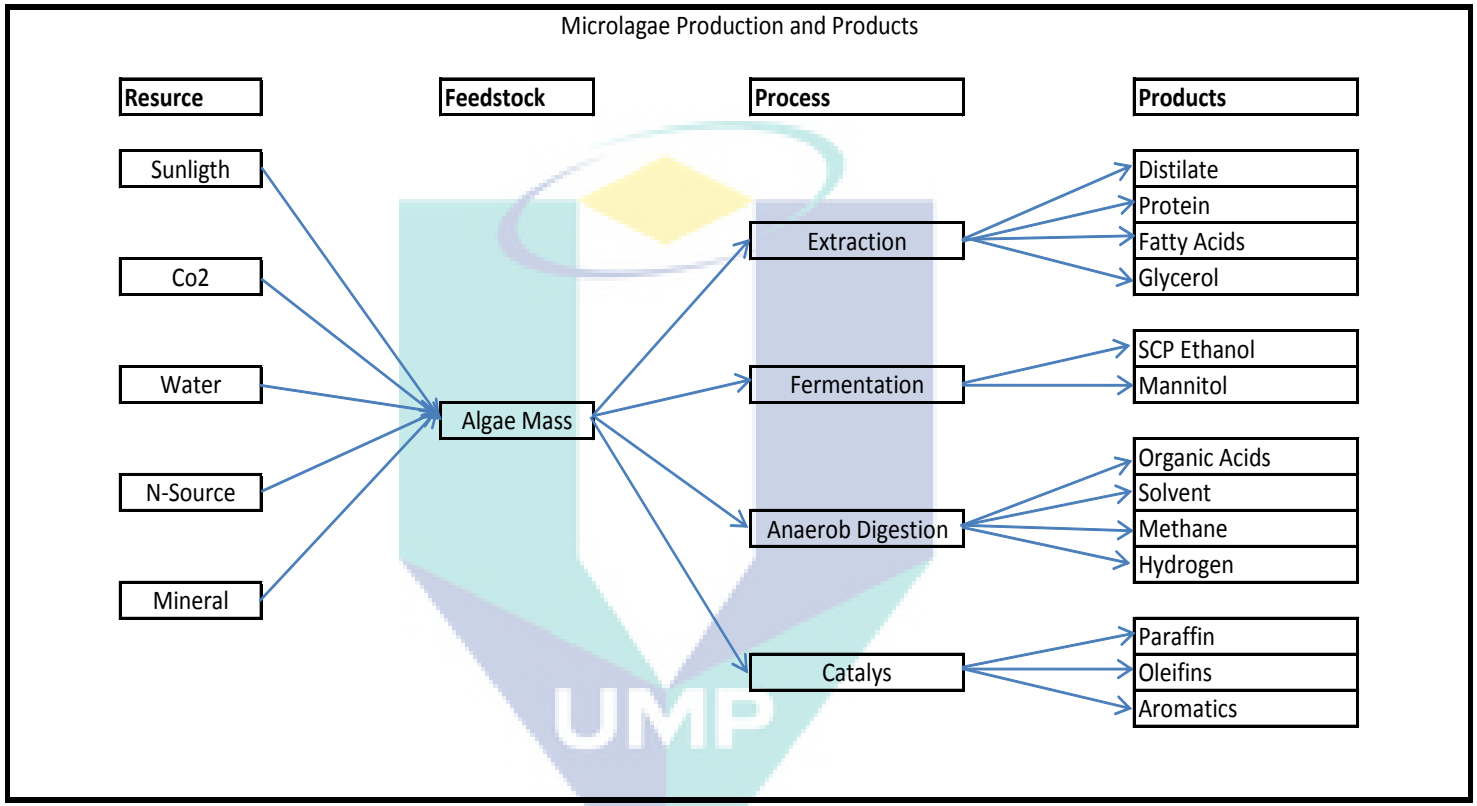
2.1.3.3.c Biodiesel

Some microalgae are relatively high in lipids. This makes some microalgae potential to be changed into biodiesel. Figure 2.4 below explains the process applied and the fuel products resulted from microalgae.

2.1.3.4 Others

Comparing to the diversity of types and chemical components of the existed microalgae, microalgae utilization is still less considerable. In the field of chemical process and environment, microalgae have been utilized in some aspects.

Microalgae are able to absorb heavy metals because of the functional constellation of carboxyl, hyrocyl, sulfudryl, amino, imodazol, sulphate, and sulfonate inside the cell wall. Moreover they also can be used as bioactive compound for they have the ability to produce bioactive secondary metabolite for self-protection.



(Hill and Feinberg, 1984)

Figure 2.4 Microalgae and their derivative products

Alginate compound which exists in the cell wall of microalgae makes it possible for microalgae to be used in textile industry to repair and improve the quality of textile material. This compound is able to produce heteropolysacaride resulted from the formation of monomer mannuronic acid and gulunoric acid chain. In the form of Calcium, alginate is usually used in pharmacy (to make medicine), food industry, and frozen dairy product to anticipate the formation of ice crystal. Pharmaceutical industry uses this compound as the basic material for covering capsule and tablet. Alginate is also used as biomaterial for medication technique like micro-encapsulation and cell transplantation. Moreover, some microalgae are also potential as the basic material for the environmentally-friendly plastics (biodegradable plastics). In addition, a type of freshwater microalgae named Cladophora is potential to be used as battery in the future (environment-friendly, thin and flexible).

2.2 Biodiesel Microalgae

2.2.1 History of Biodiesel Microalgae

The process of transesterification as the main reaction of biodiesel production was firstly introduced by E. Duffy and J Patrick in 1853. On 10 August 1893 in Augsbrug (Germany), the diesel engine was firstly introduced by Rudolf Diesel. Together with French Otto Company Rudolf Diesel introduced the diesel whose fuel was from nut oil in an exhibition in Paris in 1900. At that time, the kerosene was usually used to run the diesel engine. In some countries like Belgium, France, England, Italy, Portugal, Germany, Argentine, Japan and China have reported the test and the use of plant oil since 1920 until the end of World War 2. The patent of plant oil processing was released in 1973 in Brussels university namely "The transformation procedures of plant oil fuel," Patent of Belgium, 422,877.

The use of biodiesel became popular in 1980 due to the decrease of fossil fuel reserve. Besides, biodiesel is expected to be able to reduce the pollution caused by the use of fossil fuel. The idea of using microalgae for fuel is not a new one, since 1970s America with its NREL (National Renewable Energy Laboratory) has done studies on screening, genetic engineering and mass production system. Long time before it, in 1957, Golueke conducted research on processing microalgae into fuel (methane gas) using anaerobic process. Today some countries have developed microalgae as biodiesel fuel intensively. Mexico has developed microalgae culturing using open pond system massively. Similarly, Singapore has invested a big amount of money to build electricity generator plant from algae; even Italy has prepared electricity generator plant and biodiesel from algae, to fulfill the needs for electricity and fuel in Venesia port. Although it is not a new thing, Chisti and Gavirelscu (2005) argued that biodiesel microalgae has been very important because of 2 reasons namely the escalation of oil price in the world and global warming issue.

2.2.2 Biodiesel Processing

2.2.2.1 Definition of biodiesel

According to ASTM D 6751, EN14214 or IS 15607 biodiesel is defined as fuel source arranged from long chain fatty acids monoalkylester which is the derivation of plant oil or animal fat. Biodiesel has similar physical characteristic with diesel fuel so it can be used to replace the diesel oil used in the vehicles. The chemical composition of biodiesel is different from diesel fuel; in general biodiesel contains C16-C18 fatty acids methyl ester with 1-3 double bonds. This characteristic is advantageous comparing to diesel fuel in term of gas emission, sulphur level, octane number, decomposition, lubrication and machine cleaning.

Biodiesel in general is produced from reaction between alcohol and triglycerides in the plant oil. The oil, taken from the seeds, nuts or vegetables, is composed from triglycerides molecule (or known as triacylglycerol or triacyglycerides).

2.2.2.2 Process of Transesterification

Transestrification is the conversion of glycerol ester (triglycerides) to monoester of lower alcohols, typically methanol or ethanol, as shown in Figure 2.7. This process is commonly used in the detergent industry for the conversion of triglycerides into fatty acids, ester and alcohols. While either acids or bases have been used as catalysts, bases such as sodium hydroxide (caustic soda) and sodium methoxide have produced slightly faster conversion at higher conversion rates than the acids.

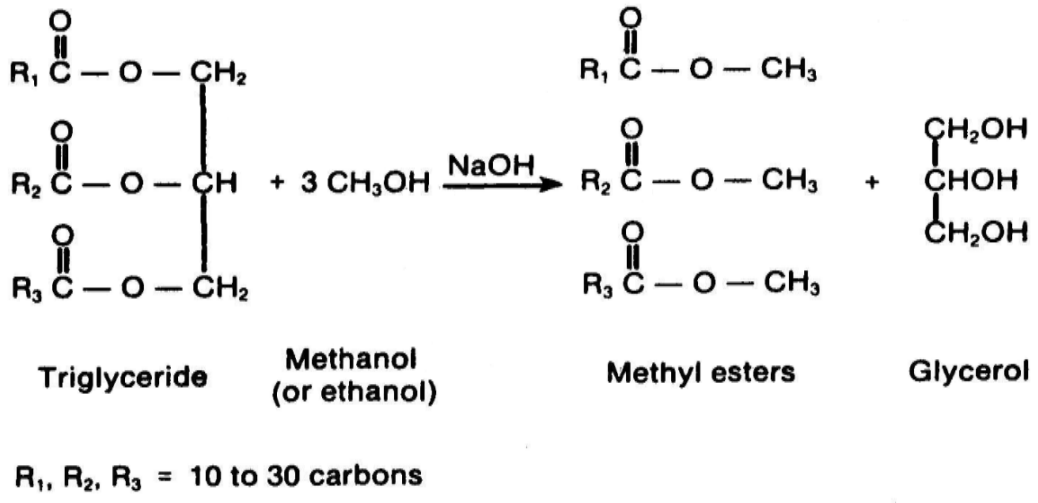


Figure 2.5 Illustration of Transestrification process

Based on the Transesterification process, the biodiesel processing can be done using four types of reaction; they are: a) Transesterification reaction using base catalyst, b) Direct Transesterification reaction using acid catalyst, c) Conversion reaction of raw oil becoming fatty acids and then continued to become biodiesel and d) Transestrification reaction without any catalyst.

The mechanisms of the break of triglyceride bounds becoming biodiesel using transesterification reaction process follow three steps; 1) Fat or plant oil has big molecule structure, composed from glycerol and 3 fatty acids, 2) Using Transesterification process, the fatty acids are moved from glycerol, and each fatty acid is then bounded by methanol. 3) The final result 1 mol glycerol and 3 mol fatty acid methyl ester (FAME) or called Biodiesel

2.2.2.3 Ultrasonic- Assisted *In Situ* Transestrification

Another alternative to the conventional process, which is considered to have potential of reducing the processing units and costs of the fuel conversion process, is the '*in situ*' Transesterification method. The *in situ* process facilitates the conversion of the biomass oil to FAME directly from the oil bearing biomass, thereby eliminating the solvent extraction step required to obtain the oil feedstock as in the conventional method.

This biodiesel production scheme could therefore aid the simplification of the fuel conversion process, potentially reducing the overall process cost, hence lowering the final fuel product costs as well (Hass, 2007). This method may be advantageous for use with microalgae, since the extraction of microalgae lipids is usually accomplished via solvent extraction and not with the use of cheaper physical extraction methods (for example expellers) as utilized for conventional oil crops.

In-situ transesterification differs from the conventional reaction in that the oil-bearing material contacts with acidified or alkalized alcohol directly instead of reacting with pre-extracted oil and alcohol. That is, extraction and transesterification proceed in one step, the alcohol acting both as an extraction solvent and an esterification reagent.In a simple way, the difference of conventional transesterification reaction and *in-situ* transesterification was explained in his research report as described in Figure 2.9.

Marinocovic and Tomasevic (1998) also reported that the *in situ* transesterification process done to the sunflower seeds using acidified methanol was proven to yield fatty acid methyl ester (biodiesel) significantly greater than those obtained from conventional reaction with pre-extracted seed oils.

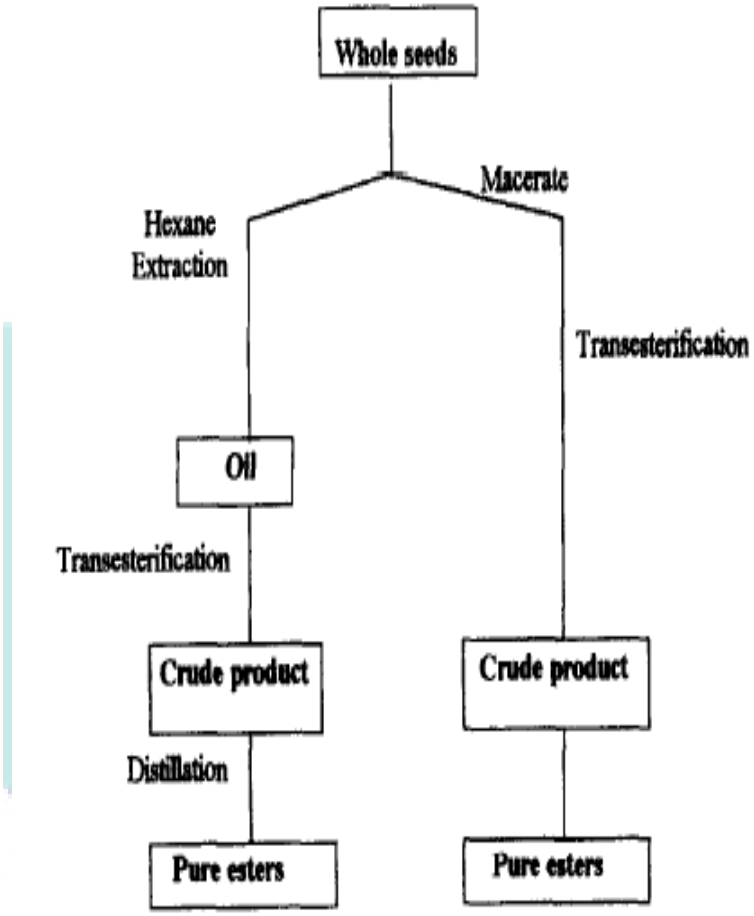


Figure 2.6 Conventional and *in situ* transesterification processes (Harrington KJ, et al, 1985)

In the last two decades, sonochemistry, the chemical reaction during ultrasound irradiation, has developed into an expanding research area. Ultrasound energy is known to produce chemical and physical effects that arise from the collapse of cavitation' bubbles. The collapse of cavitation bubbles disrupts the phase boundary in a two-phase liquid system and causes emulsification by ultrasonic jets that impinge one liquid in to the other. This effect can be employed for biodiesel production. Ultrasonic horns are the most commonly used reactor designs among the sonochemical reactors, although the cavitation effects are only observed close to the

vibrating surface. The cavitation intensity decreases exponentially on moving away from the horn and vanishes at a distance of as low as 2–5 cm, depending on the supplied energy to the equipment and on the operating frequency (Gotate, 2002).

The application of the *in situ* transesterification process using homogenous acid catalysts for biodiesel production from oil bearing biomass is not a novel one. The method was first demonstrated by Harrington and D'Arcy-Evans with sunflower seeds as feedstock. Using the *in situ* method these authors achieved an increase in biodiesel yields of up to 20% compared to the conventional process. This improvement in the biodiesel yields was considered by these authors to be attributable to the improved accessibility of the oil in the biomass by the acidic medium (Harington and D` Arcy, 1985).

2.2.2.4 Biodiesel Quality

Based on Indonesia National Standard (SNI) 04-7182-2006 and ASTM, the specification of biodiesel quality is based on some parameters which have standardized value. The function of this standard quality is to guarantee the quality and performance of resulted biodiesel so it can be used for the diesel engine. The comparison of biodiesel quality with SNI and ASTM can be seen in Table 2.4 below:

Table 2.4.The Quality Comparison of Microalgae Biodiesel, diesel oil and ASTM (Han Xu, et.al, 2006)

Parameter	**Microalgae Biodiesel**	**Diesel Oil**	**ASTM**
Densities ($kg.L^{-1}$)	0.864	0.838	0.86-0.9
Viscosities (mm^2s^{-1})	5.2	1.9-4.1	3.5-5.0
Flame point (^oC)	115	75	Min 100
Solid point (^oC)	-12	-50-10	-
Cold filter plugging	-11	-3 (max -6.7)	0 - < -15
Acid value (mg $KOH.g^{-1}$)	0.374	Max 0.5	Max 0.5
Heat value ($MJkg^{-1}$)	41	40-45	-
Ratio H/C	1.81	1.81	-

2.3 Extraction Principles

2.3.1 The Principles of Solid Liquid Extraction

Solid liquid extraction is one of the methods for preserving valuable resources in addition to protecting the environment from hazardous waste. Solid-liquid extraction is among the most commonly employed methods of separation, which appears in many industrial processes for example pharmaceutical industry, perfumes or pesticides manufacturing industries to recover active component from plants (Luque de Castro and Garcia-Ayuso, 1998; Romdhane and Gourdon, 2002).

Extraction is a separation process to separate the desired solute or remove an undesired solute component from the solid phase where the solid is contacted with a liquid phase. Two phases are in intimate contact and the solutes can diffuse from the

solid to the liquid phase, which causes a separation of the component originally in the solid. The extraction process also depends on how fast the compound will dissolve and reach the equilibrium concentration in the liquid. Solid-liquid extraction also known by variety of other names, such as leaching, washing, percolation, digestion, steeping, lixiviation and infusion but of this only one term, leaching has widespread use (Geankoplis, 1993; Ruthven, 1997; Luque de Castro and Garcia-Ayuso, 1998; Cacace and Mazza, 2003).

The simplest extraction system comprises three components: a) Solute, or the material to be extracted, b) Solvents, which may be a liquid or a supercritical fluid at process conditions, and c) Carrier or non-solute portion of the feed mixture to be separated.

For the case of countercurrent extraction and a light solvent, the flow of the materials is as shown in Figure 2.10.

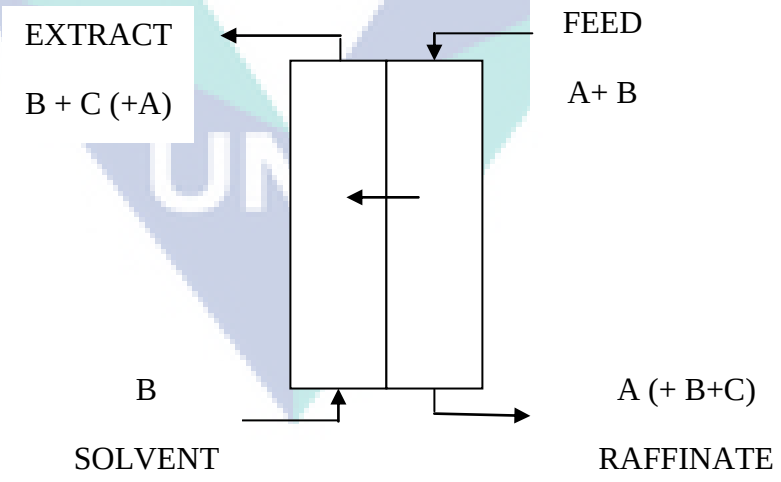


Figure 2.7 Extraction notation

where, Reffinate phase: feed stream minus extracted material, Extract phase: solvent stream plus extracted material

A = Carrier, B = Solvent, C = Solute

For such system the carrier and the solvent are essentially immiscible, while the carrier-solute and solvent-solute pairs are miscible.

2.3.2 Mass Transfer in Extraction Process

In general, the following steps can occur in an overall solid-liquid extraction process (Geankoplis, 1993; Holland, 1975): a) solvent transfer from the bulk of the solution to the surface of solid, b) penetration of diffusion of the solvent into the pores of the solid, c) dissolution of the solvent into the solute, d) solute diffusion to the surface of the particle, and e) solute transfer to the bulk of the solution.

Any one of the five basic steps may be responsible for limiting the extraction rate. The rate of transfer of solvent from bulk solution to the solid surface and the rate into the solid are usually rapid and are not rate-limiting steps and the dissolution is usually so fast that it has only small effect on the overall rate (Geankoplis, 1993; Ruthven, 1997).

The overall extraction process is sometime subdivided into two general categories according to the main mechanism responsible for the dissolution stage: a) operations that occur because of the solubility of the solute or its miscibility with the solvent e.g. oilseed extraction, b) extractions where the solvent must react with a constituent of the solid material in order to produce a compound soluble in the solvent, e.g., the extraction of metal from metalliferous ores.

In the former case the rate of extraction is most likely to be controlled by diffusion phenomena. The diffusivity is an important property affecting the diffusion

process. Smaller particle size reduces the diffusion distance of the solute within the solid and increase the concentration gradient, which increase the extraction rate. Since the path of solute to reach the surface is shorter, extraction time is reduced. An increase of temperature significantly increases diffusivity as established by the Einstein Equation

$$D\alpha(T/\eta) \tag{2.1}$$

Where T is the absolute temperature and η, the dynamic viscosity coefficient. The increase in diffusivity due to temperature may be caused by an increase of the internal energy of the molecules and thus their mobility and a reduction of the dynamic viscosity coefficient (Cacace and Mazza, 2003).

As stated before, the process of solid-liquid extraction involves the transfer of material from one phase to another. Therefore it is categorized as a mass transfer process. Mass transfer means that the redistribution of molecules under the influence of a potential driving force. The potential used to bring about this change may be a chemical potential (created by concentration differences) or other forms of potential e.g. electrical, gravitational etc (Phipps and Eardley, 1982; Gekas, 2001).

When mass is being transferred from one distinct phase to another or through a single phase, the basic mechanism is the same whether the phase is a gas, liquid or solid. The equation for molecular diffusion of mass is Fick's Law. It is written as follows for constant total concentration in a liquid (Geankoplis, 1993; Lydersen, 1983; Seader and Henley, 1998).

$$J^*_{AZ} = -D_{AB}\frac{dc_A}{dz} \tag{2.2}$$

Where J^*_{AZ} is the molar flux of component A in the z direction due to molecular diffusion in kg mol A/s.m^2, D_{AB} is the molecular diffusivity of the molecule A in B in m^2/s, C_A the concentration of A in kg mol/m^3, and z the distance of diffusion in m.

This equation is more commonly used in many molecular diffusion processes. If C_A varies to some extent, an average value is often used with equation 2.2. Rearranging equation 2.2 and integrating,

$$J^*_{AZ} = \frac{D_{AB}(c_{A1} - c_{A2})}{z_2 - z_1} \tag{2.3}$$

The general Fick's Law equation can be written as follows for a binary mixture of *A* and *B*:

$$J^*_{AZ} = -cD_{AB}\frac{dx_A}{dz} \tag{2.4}$$

Where the total concentration of *A* and *B is* in kg mol $(A + B)/m^3$, and x_A is the mole fraction of *A* in the mixture of *A* and *B*. If *c* is constant, then $c_A = cx_A$.

$$C\,dx_A = d(cx_A) = dc_A \tag{2.5}$$

2.3.3 Convective Mass Transfer Coefficient

When a fluid is flowing outside a solid surface in forced convective motion, the rate of convection mass transfer, N_A from the surface to the fluid, or vice versa can be expressed by the following equation:

$$N_A = k_c\,(c_{L1} - c_{Li}) \tag{2.6}$$

where the k_c is a mass transfer coefficient in m/s. This is an indirect measure of the resistance to transfer. The effectiveness or efficiency with large k_c values indicates an effective transfer for the reason that a low transfer resistance exists. Similarly the larger mass transfer coefficient is produced the more rapid the rate of extraction. c_{L1} is the bulk fluid concentration in kg mol*A*/m^3 and c_{Li} is the concentration in the fluid next to the surface of the solid (Sherwood and Pigford, 1952; Schweitzer, 1979).

2.3.4 Molecular Diffusion in Solid

Transport in solids can be classified into two types of diffusion (Geankoplis, 1993); a) Diffusion that can be considered to follow Fick's Law and does not depend primarily on the actual structure of the solid, and b) Diffusion in porous solids where the actual structure and void channels are important.

Diffusion in solids following Fick's Law does not depend on the actual structure on the solid. The diffusion occurs when the fluid or solute diffusing is actually dissolved in the solid to form a more or less homogeneous solution for example in leaching, where the solid contains a large amount of water and a solute is diffusing through this solution (Geankoplis, 1993; Treybal, 1980).

Generally, simplified equation is used.

$$N_A = \frac{-D_{AB} dc_A}{dz} \tag{2.7}$$

Where N_A is flux in kg mol$A$/s.m^2, D_{AB} is diffusivity in m^2/s of A through B and usually is assumed constant and independent of pressure for solids.

Integration of equation 2.7 gives

$$N_A = - \frac{D_{AB}(C_{A1} - C_{A2})}{z_2 - z_1} \tag{2.8}$$

2.3.5 Turbulent Diffusion Equations for Mass Transfer

If the fluid is agitated or mixed, transfer takes place by the relatively fast process of eddy diffusion. Conditions favorable to eddy diffusion may be maintained by special mixing devices or agitators, or by the establishment of turbulent flow (Sherwod and Pigford, 1952; Treybal, 1980; Geankoplis, 1993).

For turbulent mass transfer for constant c,

$$J^*_{AZ} = -(D_{AB} + \varepsilon_M)\frac{dc_A}{dz} \quad (2.9)$$

Where D_{AB} is the molecular diffusivity in m^2/s and ε_M is the turbulent or mass eddy diffusivity in m^2/s. Integrating equation 2.9:

$$J^*_{AZ} = \frac{D_{AB} + \varepsilon_M}{z_2 - z_1}(c_{A1} - c_{A2}) \quad (2.10)$$

The simplified equation is written using convective mass transfer coefficient

$$J^*_{AZ} = k'_c(c_{A1} - c_{A2}) \quad (2.11)$$

Where k'_c is $(D_{AB} + \varepsilon_M)/(z_2 - z_1)$

2.3.6 Extraction Techniques

There are many types of techniques to extract microalgae oil that can be used nowadays. Each of these techniques have advantages and disadvantages depending on what is the material that will be extracted. Currently, the most popular techniques had been used in many extraction activities are solvent, expeller, enzymatic, supercritical, ultrasonic, osmotic and electromechanic.

2.3.6.1 Solvent Extraction Method

This type of extraction is frequently used in the plant-oil capturing process. This process is also called soxhlet extraction. Some frequently used solvents are n-hexane, petroleum ether, ethanol and benzene. In selecting the solvent, things to consider are polarity level, toxicity level, volatility and price. Algae oil can be extracted using some chemical solvents. Benzene and ether can be used in this process, but the popular solvent is hexane because the price is cheaper and the polarity level is smaller. Some of the chemical materials are dangerous so it needs to be very careful in using them. Besides, some of them are flammable and explosive and some others are carcinogenetic which can cause cancer.

Some materials are safer in term of toxicity, for example alcohols like ethanol or methanol. Ethanol is chosen because it has longer carbon chains than methanol. The longer the carbon chains, the smaller the polarity level is. In that way it is easier to extract the algae oil which also has small polarity level. Moreover, using alcohol solvent, the proteins from the residue of microalgae biomass can still also be taken. Then, they can be used for health supplement food. Meanwhile the carbohydrates can also be taken and used for bioethanol and bioplastic.

Some of the things that need to be considered using this method are the high yield (around 95% of the total lipid content), ease of processing, and the low cost. However, in the implementation for the big scale, this method has some weaknesses, for example, it needs longer time (16-24 hours using 4-6 circulation per hour), it consumes enormous solvent, it requires the process of retaking the solvent and finally it uses enormous heat energy.

There is also another solvent method using different technique i.e. soaked-stirred extraction. In this model, the solvents can be used independently or combined with variety of solvents to become one. The example is combining chloroform-methanol and water.

2.3.6.2 Expeller extraction method

Expeller method is done by hydraulic pressing or squeezing/screwing. Before pressing the microalgae is washed and dried. The algae oil yielded using this method is 70-75%. In the commercial scale, to improve the percentage of algae oil taken, this expeller technique is combined with the use of benzene or cyclohexane or hexane. The solvent is added to take the remaining oil existed in the algae after pressing. The effectiveness of this combination process is proven by the fact that 93% of the algae oil can be extracted.

Some advantages of using this method are it is a cheap technology, the operation of the tools is easy, and it needs a relatively small amount of energy. However, the result is low if there is no solvent combination. Moreover, when the solvent combining is applied, the process of solvent-removal needs to be done.

2.3.6.3 Enzymatic Extraction Method

The process of enzymatic extraction is one of methods found and used to take the oil from the plants. This method has widely applied to extract plant oil from, for example, soybean, coconut, olive, sunflower, avocado, cotton seeds, canola and corn.

Enzymatic method, using enzyme to degrade the cell wall using water as the solvent, is a trusted method and can be applied in extraction process of plant oil. Using this method, the oil produced is relatively good (79% from the total lipids). The enzymes used are alcalese or amilase. Some of the things that need to consider in using this method are: the incubation time (1-3 hours), acidity level (approximately pH 4-9), and temperature (approximately 40-60^{o}C).The benefits of using this method are: some other components can be taken from the residual of the biomass and the process is environmentally friendly. But, the cost is far more expensive.

2.3.6.4 Super critical method

This method is a new method which is relatively efficient in the oil-capturing process. This process requires temperature and pressure controls in supercritical condition. Supercritical method uses liquid as well as CO_2 in which pressure and the critical temperature are applied to take the oil from the solid body.

Using this method, 100 % of the algae oil can be extracted, meaning that all oil contained in the microalgae can be taken.

The weakness of this method is it requires special equipment and treatment due to its complexity. Besides, the cost of the equipment is expensive and so this method is rarely used in the commercial scale.

2.3.6.5 Ultrasonic extraction Method

This method is also a new extraction method. This method requires ultrasonic waves using the wave frequency of 20-40 kHz as the extractor means. The use of ultrasonic causes the cavitation bubbles in the solution. When the bubbles burst near the cell wall, shock wave and will occur and cause the heat increase and break the cell wall of the microalgae. And then, the oil yielding will be taken by the solvent.

The use of ultrasonic waves is to speed up the break of the cell wall in the extraction process. Meanwhile, the lipid picking process from the body of the broken cell is done by the solvent. The most frequently used solvents are n-hexane, benzene and alcohol. Ultrasonic wave is advantageous in term of short extraction time and low temperature i.e. approximately 5-13 minutes and 20-40^{O}C. The lower temperature and shorter time are advantageous because they decrease the amount of extraction energy used in the process. However, it also requires solvent-removal process.

2.3.6.6 Osmotic Method

Osmotic method usually used strong acid with high molarity level. The high level of acid in the solvent influences the osmotic pressure on the cell wall of microalgae. As the result, water inside the cell will move to the solvent. This movement will cause the cell shrink or called lyses, causing the oil is extracted from the oil cavity inside the cell. The strong base solvent can also be used as the osmotic

extractor, but gelling (the formation of gel) is frequently found due to the existence of protein in the algae which possibly tends to be base.

In biology, a hypotonic solution has the lower osmotic pressure of two fluids and also describes a cell environment with a lower concentration of solutes than the cytoplasm of the cell. Given a cell placed in a hypotonic environment, osmosis causes a net flow of water into the cell, causing swelling and expansion. This swelling can cause the cell to burst.

2.3.6.7 Electro-mechanic Method

Basically, this type of extraction uses electricity power which causes mechanical pressure on the cell wall causing the break of the cell and releasing the oil from the cell. The principle of this method is membrane cell of microalgae has high level of obstruction, when the electric field is directed right to the cell wall of the microalgae, the induction current between surfaces will occur in each side of the membrane cell.

The electric field applied will result in 1 of 2 of the following things, or even both may occur. First is electroportation, due to the electricity, the cell wall will be broken and make holes on the cell wall. Second is electrodistention, when the power of electrostatic appears, the stretches will occur or the algae cell will be pressed. These will make the break of the cell wall.

2.4 Ultrasonic Assisted Method

2.4.1 Theory of Ultrasonic

Ultrasound uses similar approach just like other sound in describing its acoustic energy. Both potential and kinetic energies are associated with vibration. The potential energy, E_p is

$$E_p = \frac{p^2 V_O}{2C^2 \rho} \tag{2.13}$$

Where

ρ = density of medium

C = velocity of the sound in the medium

P = sound pressure

V_o = steady state volume

The kinetic energy, E_k is simply

$$E_k = 0.5p\, u^2\, V_o \tag{2.14}$$

Where

u = the velocity of displacement.

The total energy associated with the volume V_o is the sum of Ep and Ek. Energy is transferred from one vibrating particle to the next and acoustic energy travels through the medium as a wave (Porges, 1977). The propagation velocity of sound waves, C in a liquid is given by:

$$C = \left(\frac{E}{\rho}\right)^{0.5} \tag{2.15}$$

Where E is bulk modulus of elasticity of the liquid and ρ is it density.

During the propagation of the sound wave, the particles of the medium are displaced from their rest or equilibrium positions. Sound generally travels in longitudinal waves in which the small local particle displacement takes place in the same direction as the wave movement. Most sound waves can exist in very viscous liquids, but their importance in acoustic is chiefly limited to sound waves in solids (Beyer and Letcher, 1969).

The term ultrasonic or ultrasound, which is a branch of acoustics, refers to vibratory waves at high frequencies, beyond human hearing. The upper threshold of normal human hearing is around 18kHz. The only distinction between audible sound and ultrasonic is that the latter cannot be detected by the human ear. The theory of ultrasonic propagation is thus exactly the same as that of audible sound (Gooberman, 1968). Ultrasonic can be generated via various types of transducer e.g. piezoelectric, magnetostrictive and mechanical oscillators. The common type of transducer for use at ultrasonic frequencies is piezoelectric. For piezoelectric transducer, an ultrasonic generator energizes the transducers by transforming an electrical energy from the power source to the transducer at the desired frequencies. When the transducers receive the signal they respond by changing shape as long as the signal is applied. The electrical energy then is converted into mechanical energy. By an appropriate choice of material and the thickness of the element, the frequency of the sound wave can be varied, whether in the audible on ultrasound range (Beyer and Letcher, 1969; Rose, 1979; Mason, 1990).

In practice, three ranges of frequencies are reported for three different uses of ultrasonic (Blitz, 1971; Ince *et al*., 2001): a) High frequency, or diagnostic ultrasound (2-10MHz). b) Medium frequency, or sonochemical-effect ultrasound (300-1000kHz) and c) Low frequency or conventional power ultrasound (20-100kHz)

2.4.2 The Application of Ultrasonic

It has been recognized for many years that power ultrasound has great potential for uses in a wide variety of processes in chemical and allied industries. The main application of ultrasound during World War I and II was in the use of sonar. After that time, there has been an increase of ultrasound applications in industry, medicine and in the consumer market. One of the major long established industrial applications of power ultrasound is for cleaning and it has proven to be an extremely efficient technology (Mason *et al*., 1996).

In recent years, there has been an ever-increasing interest in high intensity ultrasonic waves in liquids. This interest is the results of the large number of practical ultrasonic application connected with the effects of ultrasound on materials-made possible through the use of ultrasonic vibrations of medium and high intensity. Power ultrasound can be introduced into a system by either: a) Dipping the vessel containing the reaction mixture into a tank containing a sonicated liquid (most generally water) b) Using a reaction vessel whose walls are subjected to ultrasonic vibrations, and c) Immersing a source of power ultrasonic into the reaction medium itself

High intensity applications of ultrasonic are those which produce changes in or effects on the media, or the contents of the media, through which the waves propagate. Various mechanisms may be activated by the ultrasonic energy to promote the effects. Most of the effects probably can be related to the following (Blitz, 1971; Rozenberg, 1973; Ensminger, 1988; Toma *et al*., 2001): a) Heat: As ultrasound progresses through a medium, energy lost to that medium in the form of heat. Losses vary according to the nature of the medium. b) Stirring: Intense ultrasound will produce violent agitation in a liquid medium of low viscosity and disperse material by currents of liquid or accelerations imparted to the particles. c)

Cavitation: Many of the effects associated with ultrasonic occurs in the presence of cavitation. d) Chemical effect: Chemical activity, especially oxidation reactions, may be accelerated sometimes many folds, under the influence of ultrasonically produced cavitation. The effects have been variously attributed to heat and to mechanical rupture of chemical bonds. In some cases, the effect is a result of mechanical mixing or of dispersion of saturated layers that ordinarily form at an interface between the participants in the reaction. e) Mechanical effects: Stresses developed in an ultrasonic field can cause ruptures to occur in materials. They may also cause relative motion between surfaces which produces selective absorption of these surfaces, as in ultrasonic bonding of materials. Stresses developed in cavitation bubble walls can cause severe erosion of surfaces. f) Electrolytic effects: There are indications that when two metals separated on the electrolytic scale by even a small amount are exposed to intense ultrasonic irradiation in water, an accelerated galvanic action may be induced which causes electrolytic corrosion. g) Diffusion: Ultrasonic energy promotes diffusion through cell wall, into gels and through porous membranes. h) Vacuum effects: During the low pressure phase of each cycle, boiling in liquids may be induced and fluids may be drawn into tiny pores and i) Cleansing: Sometimes the observed effects may result from acoustically eroding a protection coating from a surface so that reactions between two materials may occur that would not be possible otherwise.

Pinto *et al* (2001) found that ultrasonic had been applied to extract organic compounds from different matrices and reported recovery efficiencies using ultrasonic extraction were equal to or better than using soxhlet extraction. The author concluded that shortening of the extraction time is due to an increase of both pressure (which favors penetration and transport), and temperature (which improves solubility and diffusivity).

Luque-Garcia and Luque de Castro (2003) claimed ultrasonic extraction is an effective method for extracting a number of heavy metals from environmental and industrial hygiene samples. In many cases, it provides quantitative recovery of metals and replaces drastic preparation procedures that would otherwise require the use of concentrated acids and the application of high temperatures and/or pressures (i.e. hot plate and/or microwave extraction).

Romdhane and Gourdon (2002) reported for over 50 herbal species, ultrasound has been found to produce a greater yield at lower temperature together with a shorter extraction time and in some cases better selectivity. The effect of ultrasonic waves on vegetal material breaks the cells and releases the cell's contents into the extraction medium.

Vinatoru *et al*, (1997) found that ultrasonic improved the yield of oil extracted which was obtained in a substantially shorter time even in the case of indirect sonication. The most probable mechanism for the ultrasonic enhancement of extraction is the intensification of mass transfer and easier access of the solvent to the cell material of the seeds. This is in accord with the greater yield of oil through direct rather than indirect sonication since much greater ultrasonic power is introduced in the former process.

2.4.3 Ultrasonic Aided Extraction Process

Ultrasonic method is a method that uses ultrasonic waves namely acoustic waves using the frequency higher than 16-20 kHz (Suslick, 1988). The character of ultrasonic is *non-destructive* and *non-invasive*, so it can be easily adapted to various applications. One of the advantages of using this method is to speed up the extraction process (Kuldiloke, 2002). This is proven is a study done by Cameron *and* Wang (2006) concerning the extraction of corn essence/starch. He mentioned that the corn

starch bath gained from ultrasonic process for 2 minutes is about 55,2-67,8 %, relatively the same with the one gained from the water boiling for 1 hour, i.e. 53,4%.

Using the ultrasonic and organic solvent, the extraction process of organic compound of plants and seeds goes faster. The cell wall is broken using ultrasonic waves so the each component inside the cell goes out easily (Mason, 1990). The works of ultrasonic method in extraction are as follow: the ultrasonic waves resulted from local ultrasonic generator from the micro cavities surround the extracting material, so the material is heated and then it releases the compound extract. There are double effects resulted from this process: the mess up of the cell wall to release the compounds inside the cell and the local heating of the solution and the increase of the extract diffusion. Kinetic energy passed through the whole part of the solution, followed by the emergence of cavitation bubbles on the wall or the surface, so that the mass transfer between the surface solid-liquid increase. The mechanic effect increases the penetration of the liquid to wall of the membrane cell, support the release of cell component, and increase the mass transfer (Keil, 2007). Liu *et al.* (2010) state that ultrasonic cavitation results in a break power that will break the cell wall mechanically and increase the material transfer. Some benefits of using ultrasonic technology are and its application to various starch and polysaccharide are (Lida, 2002): 1) the ultrasonic process does not need addition of chemical material and other additional material, 2) The process is quick and easy, t is low cost. 3) The process does not cause significant change on the chemical structure, particles and compounds of the materials. Factors influencing the ultrasonic ability to emerge the cavitation effect applied to the food products are: ultrasonic characteristics such as frequency, intensity, amplitude, ultrasound characteristics, like frequency, intensity, amplitude, power, product characteristics, (such as viscosity, surface pressure) and surroundings such as temperature and pressure (Williams, 1983).

The specific mechanisms to ultrasonically enhance separation processes basically depend on the cavitational effect. Since the ultrasonic extraction is tied closely to the action of ultrasonic in liquid, it will first be necessary to review briefly the present general concept of cavitation.

Cavitation takes place in a liquid when it is subjected to rapid alternating pressures of high amplitude. Ultrasonic is transmitted through a fluid as a wave consisting of alternating compression and rarefaction cycles. During rarefaction the negative pressure developed by power ultrasound is strong enough to overcome the intermolecular forces binding the fluid and tear it apart producing cavitation bubbles (Mason, 1990). During the compression cycle (high pressure), each bubbles undergoes a collapse or implosion, usually occurring in less than one microsecond. Micro-size bubbles which range in size from infinitesimal to visible (40µm and up) form and grow due to the alternating positive and negative pressure waves in a solution (Stephens and Leventhall, 1974).

Figure 2.18 visually described the mechanism of bubbles growth followed by its collapse during cavitation induced by ultrasound.

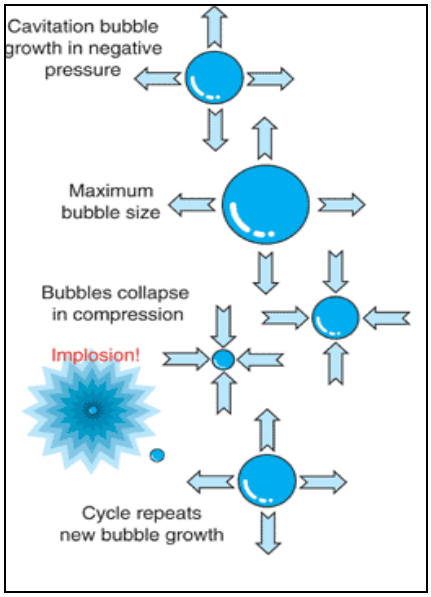


Figure 2.8 Mechanism of ultrasonic cavitation (Muralidhara, 1986)

Theoretical analysis bubbles in liquid leads to a conclusion that in order for a given bubble of radius R to exist in a liquid for a prolonged period, the internal pressure in the bubble must be equal to the external pressure. If the pressure outside the bubble becomes greater than internal pressure, the bubble will collapse (due to the condensation of vapor, or to the dissolving of gas); if it is less, then the bubble will expand (Beyer and Lecther, 1969).

Whenever a sound wave is passed through a medium the acoustic pressure supplied will be in addition to the normal atmospheric pressure already present in the liquid such that on compression the total liquid pressure will be:

$$P_L = P_h + P_a \qquad (2.16)$$

Where P_h = normal atmospheric pressure, P_a = acoustic pressure
Whilst on rarefaction it will be

$$P_L = P_h - P_a \quad (2.17)$$

The acoustic pressure at any point is the difference between the actual pressure at that point in the presence of the sound and the pressure that would exist at that point, under identical conditions, in the absence of any sound (Mason, 1990). Depending on the amplitude of the acoustic pressure, cavitation bubbles generated will fill either by gases already dissolved in the liquid, in which cases the phenomenon is sometimes called gaseous cavitation or pseudocavitation, or, in the absence of such dissolved gases, by the vapor of the liquid itself (vapor cavitation or true cavitation). The threshold for real cavitation is higher than for pseudocavitation (Stephens and Leventhall, 1974).

The cavitation threshold (the minimum acoustic pressure at which cavitation appears) depends upon frequency. The cavitation threshold becomes higher as the frequency increases. The rise in the threshold becomes significant at frequencies of about a few megacycles (Mason, 1990).

The presence of cavitation can be observed visually as a foggy cloud of bubbles in the ultrasonic field. At ultrasonic frequencies and the high intensities, the appearance of cavitation is accompanied by a hissing sound, like a tea kettle beginning to boil. This is related to the collapse of the cavitation bubbles, which causes a loud noise. The bubbles collapse will cause shock waves to be radiated from the sites of the collapse. This process would increase the temperature and also pressure in the process.

The temperature of medium in a sound field varies by two factors. The first is that the sound wave is absorbed, its energy losses thereby changing the temperature

of the medium by an amount ΔT_1. The temperature increase in this case can be calculated from the heat balance equation (Rozenberg, 1973):

$$Q_{loss} = c_v \rho \Delta T_1 x = I_o (1 - e^{a'x}) \quad (2.18)$$

In which,

$$a' = \frac{\left(\frac{4}{3}v + \chi\right)\omega^2}{2\rho C^3} \quad (2.19)$$

where,

ρ	=	Density of the medium
c_v	=	Specific heat of the medium
x	=	Path travestied by the sound wave
I_o	=	Intensity of the sound wave
χ	=	Second viscosity coefficient
C	=	Velocity of the sound in the medium
ω	=	Cylic frequency of the sound wave
v	=	Viscosity of the medium

The second factor responsible for heating up (ΔT_2) is the adiabatic compression of the medium in the sound field.

$$\Delta T_2 = \frac{C\beta * TV}{c_p} \quad (2.20)$$

Where c_p the specific heat at constant pressure, V is the acoustic particle velocity amplitude, and $\beta *$ is the thermal expansion coefficient of the medium.

In extraction processes, the good results obtained with ultrasound are also linked to the increase of the diffusion coefficient which controls the transfer of the solute to the solvent cause by the increase of temperature of the medium (Muralidhara, 1986; Schweitzer, 1979).

The ratio of the diffusion coefficient D_{ac} in the sound field to the diffusion coefficient without the field, D is equal to (Rozenberg, 1973):

$$\frac{D_{ac}}{D} = \frac{T + \Delta T}{T} \tag{2.21}$$

The most probable cavitational effect for the extraction enhancement is the intensification of mass transfer and easier access of the solvent to the solid particles. Shock wave released by the collapse of the cavitation bubbles produce energies that causes the particle disruption together with a good penetration of the solvent into the solid particle through the ultrasonic jet (Sukla *et al*., 1995). This is in accord with the greater yield of oil when high ultrasonic intensity was applied with longer extraction time since much greater ultrasonic power is introduced to the mixture.

Particle disruptions dramatically increase solid surface area, and the solid particle becomes more exposed to the solvent extraction process. Furthermore, disruptions on particle reduce the diffusion distance of the solute within the solid, so the path of solute to reach the surface is shorter; hence the extraction time is reduced. There is an additional benefit for the use of power ultrasound in extractive processes which results from the transition of laminar to turbulent in extraction mixture. The bubbles collapse in the positive pressure cycle during cavitation produce turbulent conditions. The rate of mass transfer increases with increased turbulence that promotes eddy diffusion and reduces the thickness of the boundary layer (Rozenberg, 1973; Mason, 1991).

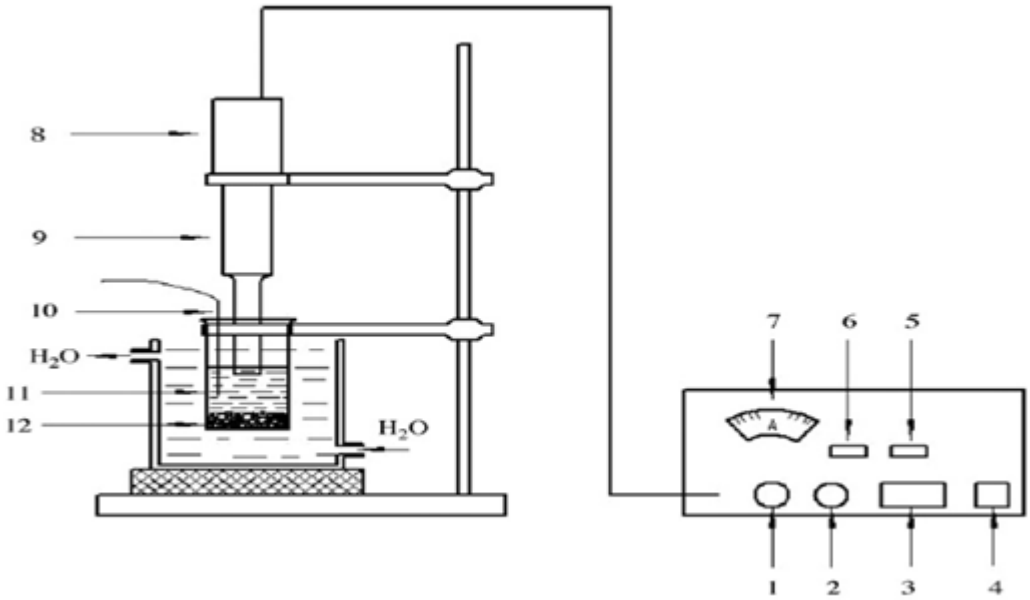
Basically, mass transfer occurs by two basic mechanisms (Seader and Henley, 1998): a) *Molecular diffusion* by random and spontaneous microscopic movement of individual molecules in a gas, liquid or solid as a result of thermal motion, and b) *Eddy* (turbulent) *diffusion* by random macroscopic fluid motion. Molecular diffusion is extremely slow, whereas eddy diffusion, when it occurs, is orders of magnetic more rapid.

Eddy diffusion increases the rate of material transfer through the ability of the natural convection that washes off the concentration build-up of solute on the surface of the particles. This will reduce the thickness of resistance near the surface of the increment of the concentration gradient, the reduction of the resistance will allow the material transfer to occur freely in the classical extraction process, and the mechanism is via normal diffusion which requires substantially longer extraction times.

2.4.4 "Vibration horn" Ultrasonic system

There are some types of reactor combinations of ultrasonic waves, namely system vibration hors system,batch, double frequencies creep, triple frequencies creep, batch system using longitudinal vibration, homogenizer, high pressure, high speed homogenizer and plat orifice (Gogate *et al*, 2006).

One of the most frequently used ultrasonic systems is vibration hornultrasonic. It uses waves transmitted with the frequency of 16-30 kHz and force up 240W. The width of the irradiation plate/ surface/ depends on the sinking depth of the vibration horn and can be used to arrange the intensity of irradiation. The configuration of this system can be used to destroy the tissue of the plant cell, to support homogenization, to increase the rapidity of the chemical reaction. The equipment, as can be seen in Figure 18 (Shan Zhang, S., at al 2008), consists of generator of waves, frequency controller, amplitude controller, and vibration horn.To support the vibration horn, static can be used (Susilo, 2007).



1	Amplitude controller	7	Electric current meter
2	Frequency controller	8	Ultrasonic transducer
3	Time presetting	9	Ultrasonic probe
4	Power switch	10	Temperature meter
5	Start button	11	Solvent (ethanol)
6	Pause button	12	Material (algae powder)

Figure 2.9 Ultrasonic Probe

2.5 Biodiesel Microalgae and Industrial Opportunity

The whole process of microalgae biodiesel processing can be seen in Figure 4.18 (Nick Sazdanoff, 2006). Biodiesel (FAME) from the microalgae nannochloropsis sp has the same chemical composition as FA component in the oil, dominated by Octadecenoic acid (C18:1) methyl ester, Dedecanoic acid (C12) methyl ester, Hexadecanic acid (C16:0) methyl ester, Tetradecanoic acid (C14) methyl ester, Octadecadinoic acid (C18:2) methyl ester and Octadecanoic acid (C18:0) methyl ester. In a previous report (Knothe, 2008), palmitic, stearic, oleic, and linolenic acid were recognized as the most common fatty acids contained in biodiesel.

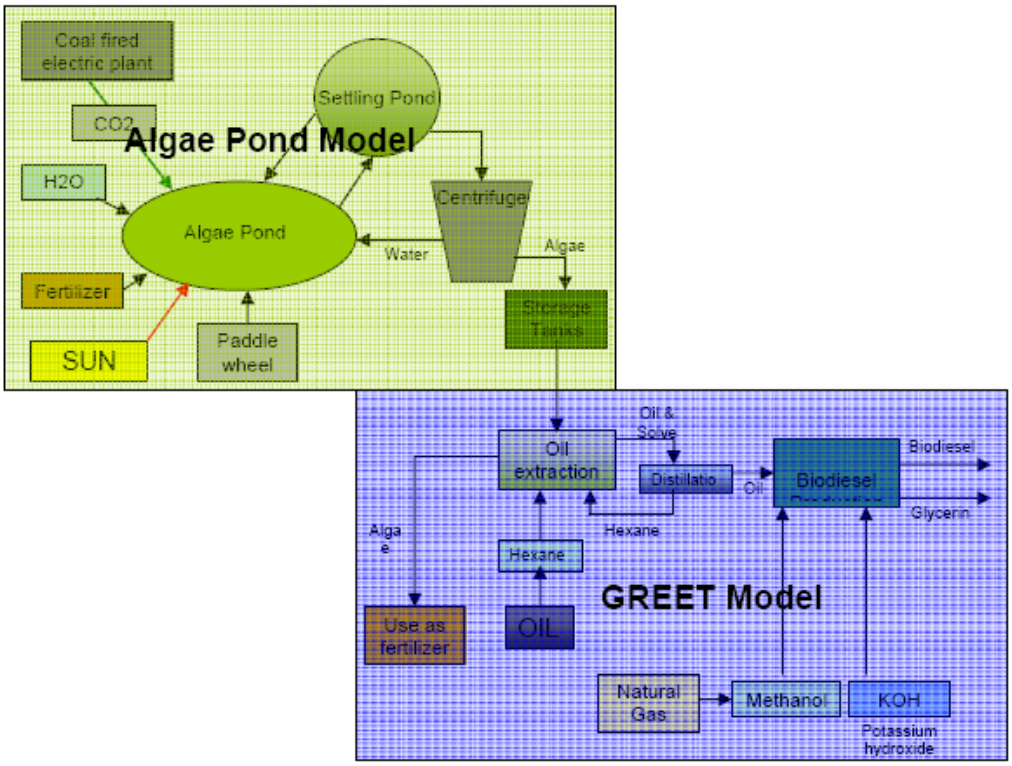


Figure2.20 Schematic of Microalgae to Biodiesel Fuel.

Oils with high oleic acid content have been reported to have a reasonable balance of fuel properties (Rashid *et al*., 2008). These fuel properties consist of ignition quality, combustion heat, cold filter plugging regions (Stournas *et al*., 1995). Among the tested microalgae species, *Nannochloropsis sp*. shows the highest oleic acid content, making it the most suitable for the production of good quality biodiesel.

In the industrial scale, the use of ultrasonic is limited to the food industry (Mason, at al, 1996). Ultrasound has been recognized for its potential industrial application in the phyto-pharmaceutical extraction industry for a wide range of oil extracts (Vinatoru, 2001).

In the 2010 Algae World Europe Industry Survey, most industry participants believe algal production will focus on three biofuels; biodiesel, JP-8 jet fuel and ethanol. The industry's most critical production challenges are production systems, harvest, extraction, and component separation followed by algal species selection, culture stability, quality control monitoring and contamination issues.

Future of microalgae industry research needs to drill down on production, supply chain and social and economic issues. Improved information on industry needs will support industry participants and provide critical information needed for public policy decisions and support. (Edward, 2010).

CHAPTER 3

METHODOLOGY

3.1. Overall Research

The bulk of the research works consists of some experimental laboratory activities, namely sample pretreatment which includes washing and drying sample, sample analysis, extraction and *in situ* transesterification of microalgae oil. The preliminary investigation indicated that pretreatment of microalgae sample and extraction method played an important role in the overall extraction yield. Figures 3.1 and 3.2 show the overall block diagram of the experimental works that has been carried out.

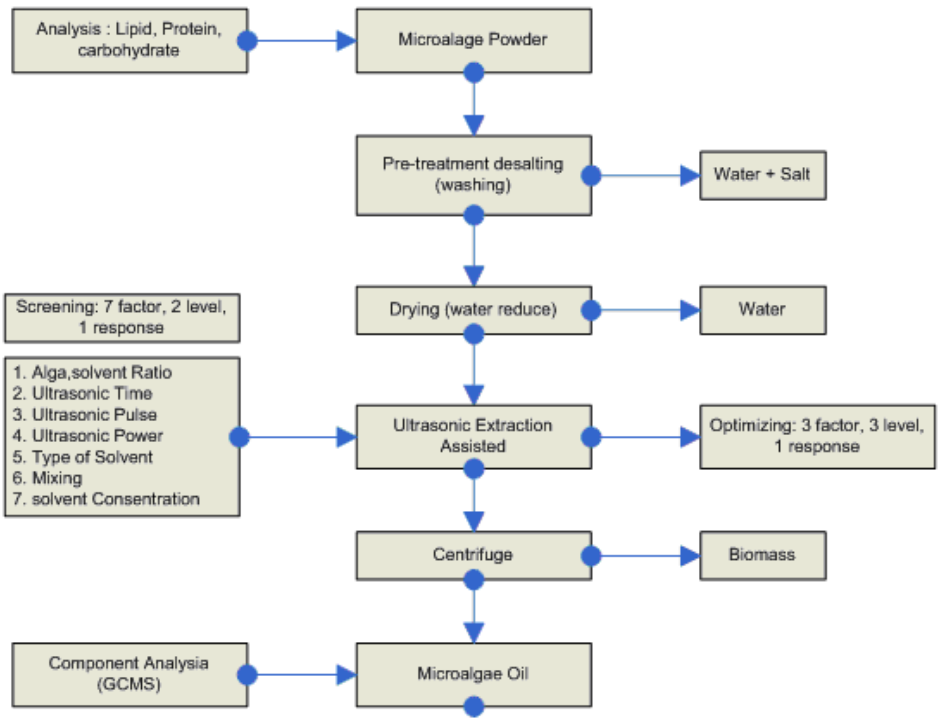


Figure 3.1 Overall methodology of Extraction research

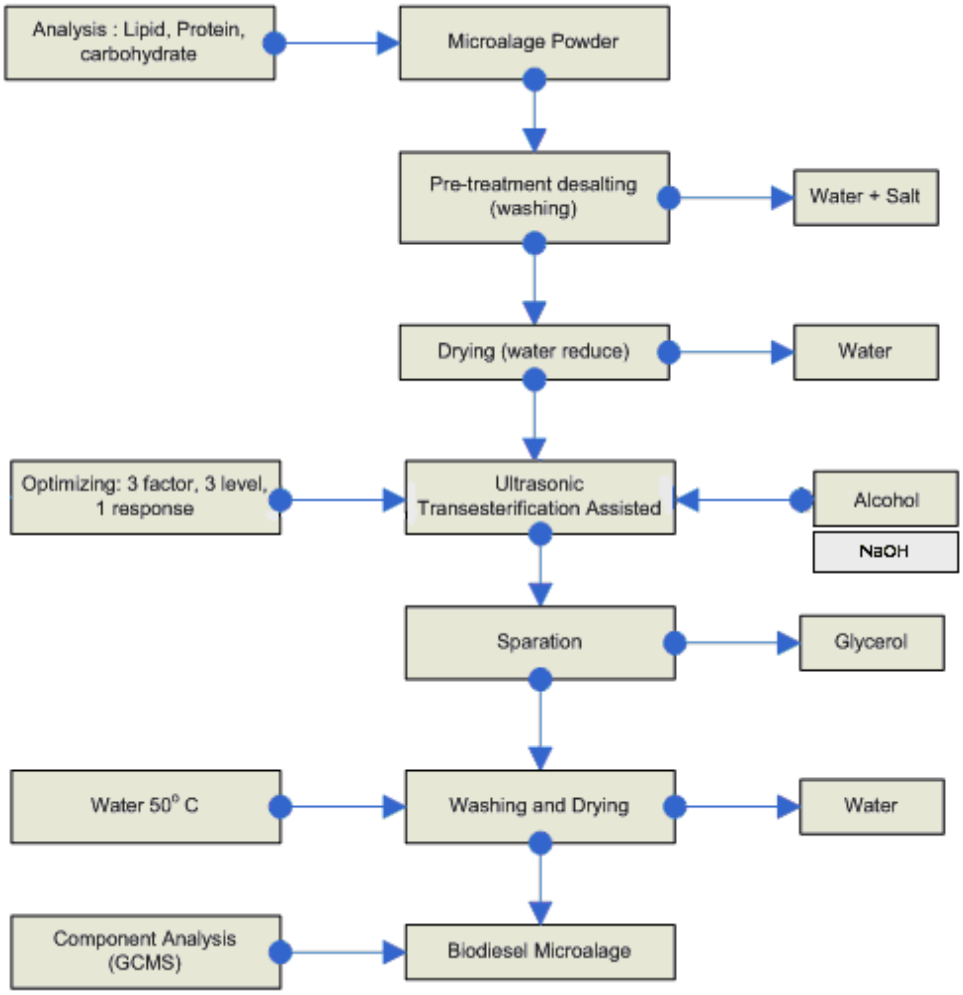


Figure 3.2 Overall methodology of *In situ* Transesterification research

Microalgae powder was placed in a thimble filter paper then put into 250 ml soxhlet. After that, ethanol was added, in ratio of 1:3 $g.mL^{-1}$. Solvent concentration was varied in the range 66% to 94%. The varying circulation is 80, 100, 150, 200 and 220 minutes, respectively.

Ultrasonic data collection was carried out by using Omni Ruptor 4000 Ultrasonic Homogenizer equipped with a power regulator, time and pulse, generator tool with dimensions of 9.90"(W) x8.75" (D) x4.60"(H) and transducer dimensions of 3.5"(Dia) x 5.0"(H) equipped with sound control chamber, working at a frequency of 20 kHz and 400 watts power consumption. Power range 0-300

Watt, and pulse time 0-15 minute set pulse and diameter of 10-100% tips on standard ¾"(19.0 mm), these tips are particularly useful for difficult cells.

The mixture of 25 grams of microalgae and 150 ml of ethanol solvent, power settings, time and pulse suit with the desired research design. The extracted liquid was separated manually. Then, filtrate was evaporated using rotary vacuum evaporator at 60^{o}C then analyzed using Gas Chromatography-Mass Spectrophotometer (GCMS). Meanwhile, the structure observation was done on the residue of microalgae biomass after ultrasonication then it was compared to the condition before ultrasonication.

3.2. Sample Preparation

The microalgae (strain type is *Nannochloropsis sp.* which was taken from *Balai Budidaya Laut (BBL*) Lampung, Indonesia) pretreatments before the extraction and *in situ* influence the quality of oil yield. These pretreatments consist of washing (to discharge salt) and drying (to reduce water). Microalgae extraction did not require grinding (to make it into small pieces/ powder) because the microalgae size was already small. Ethanol was used as solvent in the extraction process (BP Grade; 79^{o}C, BM; 46.07 g/mol).

3.2.1 Washing

Since this research used seawater microalgae strain, washing was necessary to discharge salt and other dirt. The water used was the distilled water. The water needed was about 10 times (volume) of the microalgae weight. The salt influences the solvent in penetrating the cell wall.

3.2.2 Drying

Ultrasonic extraction still requires solvent to yield the oil. Sometimes, the use of non polar solvent for the material which has a high level of water make it difficult for the solvent to penetrate the cell tissue (Akoh, 1995). Besides, water may prevent the process of in situ transesterification (Cooney, *et al*, 2009)

In this study, the microalgae, which has been washed using distilled water, was dried in the oven at 80^{o}C for 10 hours. The study on fresh water microalgae showed that the increase in drying temperature significantly influence the amount of lipid content (Widjaja *et al*, 2008). With water content of 8.32%, the use of low temperature here was aimed at preventing the damage of cell tissue and microalgae composition due to the over high temperature (Lieu, *et al*, 2009)

3.3. Sample Analysis

3.3.1 Water Content : Oven Method

The principle: Losing weight at 105^{o}C is considered as water content in the sample.

Procedures: a) Weigh carefully the 5-10 g of microalgae using the measuring bottle which weight has been known. For liquid sample, the bottle should be completed with mixer and quartz sand/folding filter paper, b) Dry in the oven of 105^{o}C for 3 hours, c) Cool in the Desiccators d) weigh, repeat this work until the weight gained is e stable

Calculation

$$Water\ Concentration = \frac{w1}{w}\ x\ 100\% \qquad (3.1)$$

In which:

w = mass/weight of the small bit before the drying process, in gram

w_1 = the mass/weight loss after drying, in gram

3.3.2 Protein Content

Principle: The nitrogen compound is turned into ammonium sulfate by thick H_2SO_4. Ammonium sulfate is decomposed by NaOH. Ammoniac released was bounded with borate acid and then tritration with blank solution

Procedures of work: a) Weigh carefully 0.51 g of the small parts, put into the Kjeldhal flask 100 ml, b) Add 2 g of selen blend and 25 ml of thick H_2SO_4, c) Heat on the electric heater or fire until boiling and the solvent turns greenish and clear (approximately 2 hours), d) Cool it, then dilute it and put it into volumetric flask 100 ml, right until the line mark, e) Pipette 5 ml of the solution and put it into the distillation tool, add 5 ml NaOH 30% and some drops of PP indicator, f) Distill for 10 minutes, as the reservoir, use 10 ml of borate acid solution 2% which has been mixed with the indicator, g) Wash the top of the cooler with distilled water, h) Titration with HCl 0,01 N and i) Do the blanko

Calculation;

$$Protein\ Concentration = \frac{(v1-v2)\ x\ N\ x\ 0.014\ x\ fk\ x\ fp}{w} \quad (3.2)$$

Where;

w = weigh of the bit

v1 = volume HCl 0.01 N sample

v2 = volume HCl blank

N = normality of HCl

fk=conversion factor for the protein off iodine general:6.25

dairy & processed products: 6.38, peanut butter: 5.46

fp = dilution factor

3.3.3 Carbohydrate Content

Principle: Carbohydrate in acid and thawed condition as well as high temperature can be broken into monosaccharide (for $\pm$3 hours). The amount of monosaccharide can be determined using Luff Schoorl Method.

Procedures: a) Weigh 2.5 g sample carefully put into Erlenmeyer 300 ml, add some boiling stones, b) Add 100ml of HCl 3%, boil below the coolant (reflux for 3 hours; calculated from the start when boiling), c) After cold, neutralize with NaOH 30% using phenolphthalein indicators as prompts (if the solution has color, use pH indicator paper), d) Filter the solution through What man filter paper40 or41,the filtrate is collected in250 ml volumetric flaks, then dilute with aquades until the mark sign, e) Pipette 25 ml of Luff Schoorl solution, put it into Erlenmeyer joint 300 ml. take 10-25 ml of the filtrate sample solution, put into Erlenmeyer Luff Schoorl, add water until the volume of 50 ml and some boiling stones, f) Reflux up to 10 minutes, calculated starting when the solution boils, g) Cool it, add 25 ml of H_2SO_4 6 N little by little (slowly). Add into it 15 ml Kl 20% solution, h) Conduct the titration using thiosulphate 0,1 N solution until the color of the solution is light yellow. Add 5 ml of amilum 0,2% solution, continue the titration using thiosulphate until the blue color disappears. Take note on the use thiosulphate, i) Do the blank titration, j) 25 ml water + 25 ml Luff Scoorl + boiling stones, reflux for 1o minutes, cool it and then do just as the sample treatment (point 7 up to 8), k) Count the amount of carbohydrate in milligram (starch/flour) using the table below.

Calculation:

The difference between ml of thiosulphate for blank titration and ml of thiosulphate for sample titration is equivalent with the amount of the starch. For example, from the reading of the table, the amount of starch is a, so:

$$Carbohydartae(\%) = \frac{250}{b} \times \frac{a}{c} \times d \times 100\% \quad (3.3)$$

In which:

a = the result of table reading of the mg starch/flour

b = volume of the sample reduced by Luff Schoorl solution

c = weight of sample (mg)

d = sample dilution factor

3.3.4 Lipid Content

Principle: Extraction of free-fat using non polar solvent.

Procedures: a) Carefully weigh 5-10 g sample, put into the paper sleeve covered with cotton on the bottom, b) Plug the sleeve which has sample in it using cotton, dry inside the oven on the temperature not more than 80^{o}C for approximately 1 hour, then put it into the connected soxhlet, c) Extract using hexane for 6 hours, d) Distill the hexane and dry the fat extract inside the oven of 105^{o}C, f) Cool and weigh, g) Repeat the drying until the weigh is stable.

Calculation:

$$\%Lipid = \frac{w - w1}{w2} \; x \; 100\% \quad (3.4)$$

Where,

w = sample weight, in gram

w_1 = weight of fat before the extraction, in gram

w_2 = weight of fat flaks after the extraction

3.3.5 Other Content (Ash)

Principle: In the process of turning the substance into ash the organic compounds are decomposed into water and CO_2, but not with the inorganic compound.

Procedures: a) Carefully weigh 2-3 g of sample into a porcelain cup (or platinum) which weight is known, for liquid sample evaporate it until dry, b) Carbonize by burning it on fire, then make it turn into ash using electric furnace at the maximum of 550^{o}C (once in a while open the door of the furnace to allow the oxygen enter), c) Cool inside the Desiccator, weigh until the weight is stable.

Calculation:

$$Ash = \frac{w1-w2}{w}\ x\ 100\% \quad (3.5)$$

where

w = sample weight before it turns into ash, in gram

w_1 = sample weight + cup after it become ash, in gram

w_2 = the weight of empty cup, in gram

3.4. Product Analysis

3.4.1 SEM Analysis

The untreated and pretreated samples were closely analyzed by scanning electron microscope model Philips SL40, Holand, which generated high resolution images of shapes of object. A small amount of sample was coated under argon atmosphere with gold prior to analysis and examined under SEM at 30kV for range magnifications between 100 to 1000 times. Figure 3.5 shows a typical SEM instrument with the electron column, sample chamber, EDS detector, electronics console, and visual display monitors.

3.4.2 GCMS Analysis

Gas Chromatography-Mass Spectrophotometer (GCMS) was used to analyze the compounds in the microalgae oil so as to compare the quality of microalgae oil produced from untreated and pretreated samples. GCMS by Agilent Technologies model 5975C was used in this study with specification as followed:

- Column : DB-WAX
- Column Length : 30 m
- Column Diameter : 0.25 mm
- Stationary phase : 5% diphenyl, 95% dimethyl polysiloxane Polyethylene glycol
- Film thickness (µm) : 0.25
- Temperature program : 60^{o}C (0.5min) 3^{o}C 230^{o}C (10min)
- Carrier gas : Helium
- Injected sample : 1 µl at split ratio 1:20

3.4.3 Free Fatty Acid and Saponification Number (Mehlenbacher, 1960)

Procedure: a) The material must be stirred evenly in liquid state when the sample is taken. Weigh as much as 28.2 + / - 0.2 g in the erlenmeyer. Add 50 ml hot neutral alcohol netral and 2 ml indicator phenolphthalein (PP) (Appendix 9), b) Titrate with 0.1 N NaOH solvent which has been standardized until the pink color appeared and does not disappear 30 second, c) The percentage of free fatty acid is regarded as oleat in most oil and fat. For coconut oil and kernel oil are regarded as laurat, while for palm oil it is regarded as palmitat, and d) Free fatty acid is stated as % FFA or acid number.

$$\%\ of\ FFA = \frac{ml\ NaOH\ x\ N\ x\ MMfa}{sample\ x\ 1000}\ x\ 100 \quad (3.6)$$

Acid number = mg KOH needed to neutralize 1 g of sample. To turn % FFA into acid number, multiply the %FFA with the factor. Factor = ((the weight of KOH /(molecule weight of fatty acid /10))

Saponification number is counted using the following formula:

$$Sponification\ Number = 28.05\ x\ \frac{blank\ titration - sample\ titration}{wight\ of\ sample} \quad (3.7)$$

3.5. Design of Experiment

3.5.1 ANOVA

ANOVA (Analysis of variance) is a general technique that can be used to test the hypothesis that the means among two or more groups are equal, under the assumption that the sampled populations are normally distributed.

ANOVA is used to uncover the main and interaction effects of categorical independent variables (called "factors") on an interval dependent variable. A "main effect" is the direct effect of an independent variable on the dependent variable. An "interaction effect" is the joint effect of two or more independent variables on the dependent variable. Whereas regression models cannot handle interaction unless explicit cross product interaction terms are added, ANOVA uncovers interaction effects on a built-in basis.

In this experiment, the factors include microalgae-solvent ratio, ultrasonic time, solvent purity, type of solvent, mixing, ultrasonic power, and ultrasonic pulse. Meanwhile, the response is the oil yield. Therefore, the experiment has 7

factors with 2 levels and 1 response as presented in Table 3.2. The combination between the factors and the levels results in 8 runs as shown in Table 3.3.

In the optimization process, a set of 3 factors with 3 levels and 1 responses shown in Table 3.4 were used in this experiment. The combination results in 15 runs as presented in Table 3.5.

3.5.2 Screening Factor

Analysis of experimental data by a Box-Behnken Design was systematically conducted using the STATISTICA version 6.0 to screen the number of experiments for examining the effect of solvent concentration, type of solvent, alga-solvent ratio, ultrasonic power, ultrasonic time, ultrasonic pulse and mixing on oil yield within empirically selected range of high (+) and low (-) levels as described in Table 3.2.

Screening was used to determine the most influential factors of the extraction process. These factors were taken from the research data of the extraction which have been done earlier.

Tabel 3.1. Factor on Design of Experiment

Factor	Symbol	Coded Level	
		High (+1)	Low (-1)
Alga-Solvent Ratio	A	1:10	1:3
Ultrasonic Time (min)	B	45	15
Solvent Concentration (%)	C	80	60
Ultrasonic Power (%)	D	50	30
Type of Solvent	E	2	1
Mixing (rpm)	F	2	1
Ultrasonic Pulse (%)	G	75	25

Type of solvent; (2) Ethanol, (1) Methanol, Mixing; (2) Yes, (1) No

Using the STATISTICA software, the combination factors were gained by 8 runs of the experiment as seen in Table 3.3.

Tabel 3.2. Variable combination for screening factor 7 factors, 2 levels and 1 response

Run	A	B	C	D	E	F	G
1	-1	-1	-1	+1	+1	+1	-1
2	+1	-1	-1	-1	-1	+1	+1
3	-1	+1	-1	-1	+1	-1	+1
4	+1	+1	-1	+1	-1	-1	-1
5	-1	-1	+1	+1	-1	-1	+1
6	+1	-1	+1	-1	+1	-1	-1
7	-1	+1	+1	-1	-1	+1	-1
8	+1	+1	+1	+1	+1	+	+1

3.5.3 Optimization

Analysis of experimental data by a Box-Behnken Design was systematically conducted using the STATISTICA software to optimize the number of experiments for examining the effect and interaction of significant factors on the result; ultrasonic power, ultrasonic time and ultrasonic pulse on oil yield within empirically selected range of high (+), middle (0) and low (-) levels as described in Table 3. 4

Tabel 3.3 Data level on optimizing

Factor	Symbols	Coded Level		
		High (+1)	Medium (0)	Low (-1)
Ultrasonic Power (%)	A	70	50	30
Ultrasonic Pulser (%)	B	80	75	70
Ultrasonic Time (min)	C	60	45	30

Using the DOE of Box-Behnken Design of industrial statistics and Six Sigma of STATISTICA version 6.0, the run of experiment was identified as seen in Table 3.5 below.

Tabel 3.4. Variable combination of experiment

Run	Power (%)	Pulser (%)	Time (min)
1	-1	-1	0
2	+1	-1	0
3	-1	+1	0
4	+1	+1	0
5	-1	0	-1
6	+1	0	-1
7	-1	0	+1
8	+1	0	+1
9	0	-1	-1
10	0	+1	-1
11	0	-1	+1
12	0	+1	+1
13	0	0	0
14	0	0	0
15	0	0	0

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Introduction

In the preliminary research, the extraction methods namely ultrasonic and soxhlet extraction have been compared. It was aimed to assure the most effective extraction method which gives the specific oil product.

The research was then continued with using the best method gained from the preliminary research *i.e.* ultrasonic. The extraction process and *in situ* transesterification of microalgae using ultrasonic are influenced by many factors. This chapter focuses on the combination of the most influential factors in the experiment. Box-Behnken Design of industrial statistics and Six Sigma of STATISTICA version 6.0 was used to screen the factors and to determine the optimum of the combinations factor or variables. In addition, the components of microalgae used were also analyzed as reference formeasurement of yield. At the end of the research, the microalgae oil produced and also the effect of ultrasonic toward the structure of microalgae after the ultrasonication were also investigated.

4.2 Microalgae Component

Microalgae components of *Nannochloropsis sp.* were analyzed to know the components. Using the method of proximate analysis, the composition of the microalgae has been known as reported in Table 4.1.

Tabel 4.1. Analysis result of Microalgae composition

No	Analysis	Result
1	Carbohydrate	0.46%
2	Water	11.18%
3	Ash	60.02%
4	Protein	4.33%
5	Lipid	4.77%

4.3 Comparison of Ultrasonic and Soxhlet Extraction

4.3.1 Soxhlet Extraction

4.3.1.1 Effect of different number of circulation (time) and ethanol purity to the oil yield

The quantitative result was measured from the volume of extracted oil per powder weight (v/m). Figure 4.1 shows the relationship between the number of circulation (time) and purity of ethanol with the oil yield (% of dried algae). The equation below shows the correlation between algae oil yield (Y, %) with the effect of circulation and ethanol purity after response surface methodology process.

$$Y = -81.1151 + 4.4377 \times A + 0.544 \times A^2 + 1.9919 \times B - 0.0084 \times B^2 - 0.0720 \times A \times B \quad (4.1)$$

Where Y is microalgae oil (%), A is circulation and B is ethanol purity.

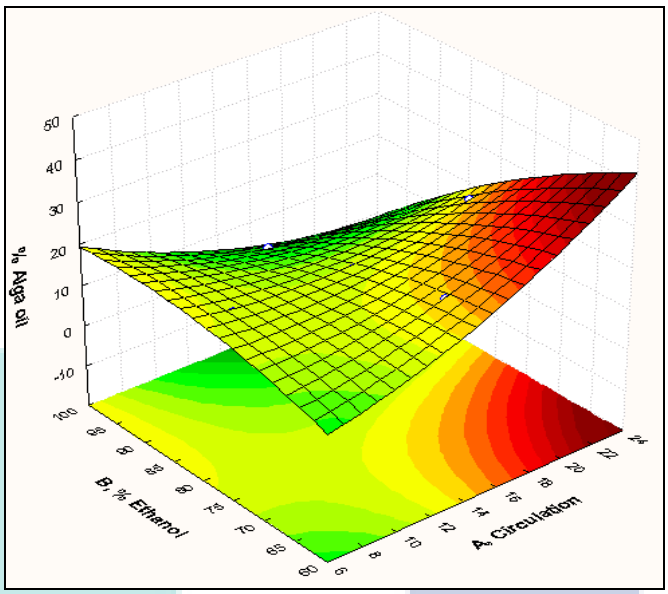


Figure 4.1 Correlation between amount of circulation (time) and purity of ethanol on the oil yield

It is clear from the figure that the more circulation time the more oil was extracted. This is in contrast with the effect of ethanol purity to the oil. The more ethanol purity, the lesser oil was yielded. This finding is in line with the result of some studies concerning ethanol solvent (Zhang *et al*, 2009; Abdullah *et al*, 2010), that there will be an increase in extraction result as the ethanol purity increase, but in a certain high ethanol purity (94%), the amount of oil extracted will decrease. The finest oil purity in this study was gained when the ethanol level was 70%, at that time the extracted oil was 8-13 ml (16-26% of algae weight). When the ethanol purities were 90 % and 94% the resulted oil was 5 ml algae oil (10 % of algae weight). It happened because the selectivity of the ethanol would firstly increase; then after a certain circumstance it would decrease. The higherpurity made other components such as phospholipids and chlorophyll were taken; it can be seen from the color of the extract which turned greener, thicker and more turbid. The high temperature also caused the oil turbidity; while the increase of circulation time immediately increased

the resulted oil. The more contact time of the material, the more oil would be extracted.

Figure 4.2 shows the relationship between experimental values versus predicted values using the model question developed (Choong, 2009). A line of unit slope, the line of perfect fit with points corresponding to zero error between experimental and predicted values is also shown in Figure 4.2. The coefficient of correlation (R^2) is 0.9770. The result in figure 2 shows that the regression model equation provides an accurate description of experimental data, indicating that it has successfully captured the correlation between the two parameters (time and purity) to the surface area.

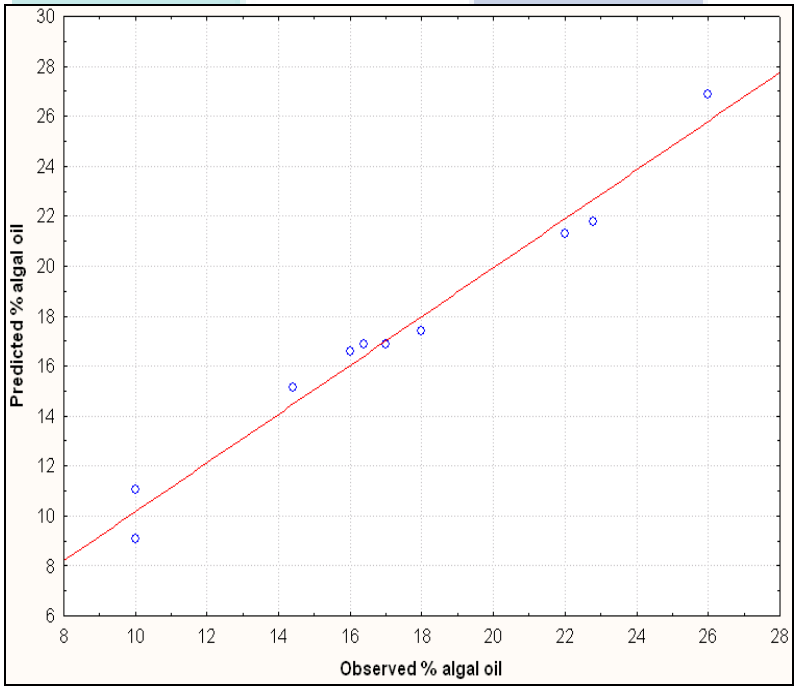


Figure 4.2 A Comparative plot between experimental and predicted surface area

4.3.1.2 Effect of time and solvent purity on the oilyield

The free fatty acid (FFA) and saponification number of the algae oil was analyzed to identify its quality of the produced oil. Saponification number is the amount of alkali needed for the saponification of some sample oil (Ketaren, 2008). For biodiesel basic material, the lower the FFA, the better oil quality is. The high level of FFA will disturb the process of biodiesel production. While the high saponification number indicates good oil quality to be used as basic material of biodiesel.

Figure 4.3 shows the correlation among the amount of circulation, solvent purity, and the FFA level gained. The long duration of circulation time made the oil contact the heat, which then influenced the oil quality. Thermal oxidation affects the oil quality. Peroxide accumulation in the algae oil at 100-115^{o}C temperature is twice bigger than that at 10^{o}C temperature; furthermore it also makes the increase of FFA and level of carbonyl oxygen in the oil (Ketaren, 2008).

Figure 4.3 also shows that there is a relationship between ethanol purity and fatty acid level. The higher ethanol purity, the higher fatty acid level will be.

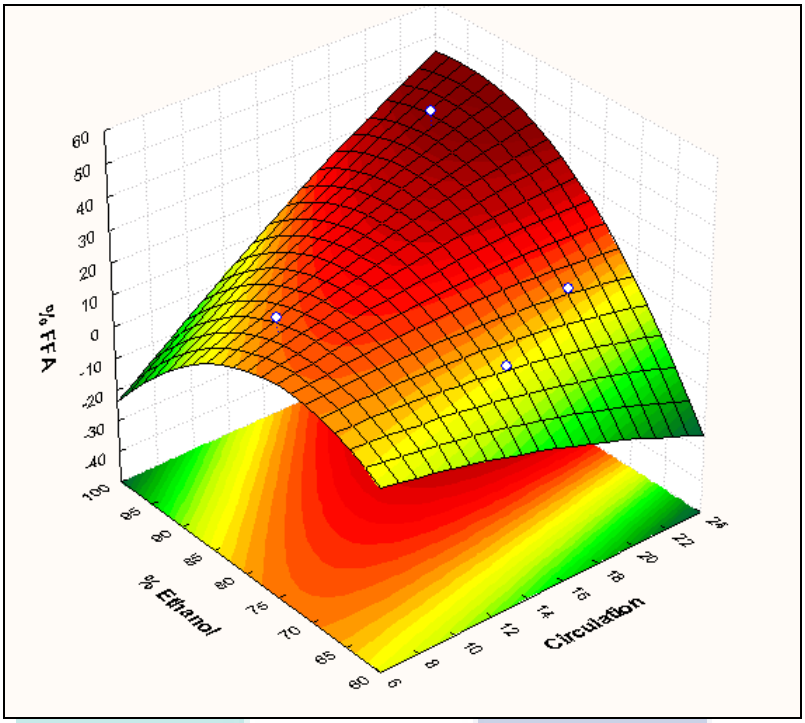


Figure 4.3 Circulation (time) vs. solvent vs. FFA

Figure 4.4 shows the relationship between the ethanol purity and the saponification number. As can be seen from the figure, at the beginning of the extraction and at low ethanol purity, the saponification number is still high. However, the saponification number tends to decrease by increasing the extraction time and the ethanol purity. This phenomenon can be explained that the temperature will increase with time that lead to the formation of longer acid chains. The acid chains formation will lower the saponification number. In addition, the use of high ethanol purity, impurities including phospholipid, wax and chlorophyll in the microalgae will also come together with the oil during the extraction process.

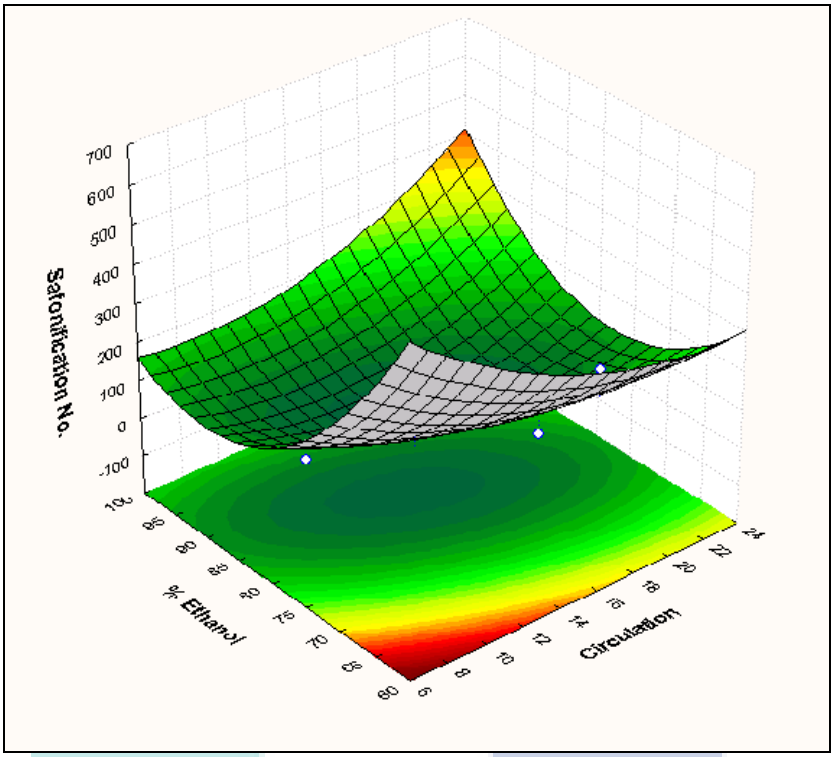


Figure 4.4 Circulation time vs. solvent purity vs. saponification number

4.3.2 Ultrasonic Assisted Extraction

4.3.2.1 Effect of temperature and solvent volume on oil yield

The effect of ethanol solvent and temperature on the microalgae extraction process was shown in Figure 4.5. Similar to soxhlet extraction, ethanol solvent also contributes to the enhancement of the yielded oil. The more solvent volume used, the more the extracted oil will be. The increase of temperature level causes a drastic increase of oil yield as shown in Figure 4.5. Temperature contributes to the rapidity of oil released from the microalgae cells as they dissolved. High temperature will catalyze the damage of the cell wall of microalgae. Therefore, the oil will be much easier to extract. However, as shown in the Figure 4.5, 96% of ethanol, which is

categorized as high purity, was not an appropriate solvent purity for soxhlet extraction of the microalgae. It is because ethanol at this purity has tendency to extract almost every solutes in the microalgae sample, making it less selective for oil extraction. This characteristic was evidenced from the oil yield extracted via this solvent medium which contained high wax, phospholipids and chlorophyll.

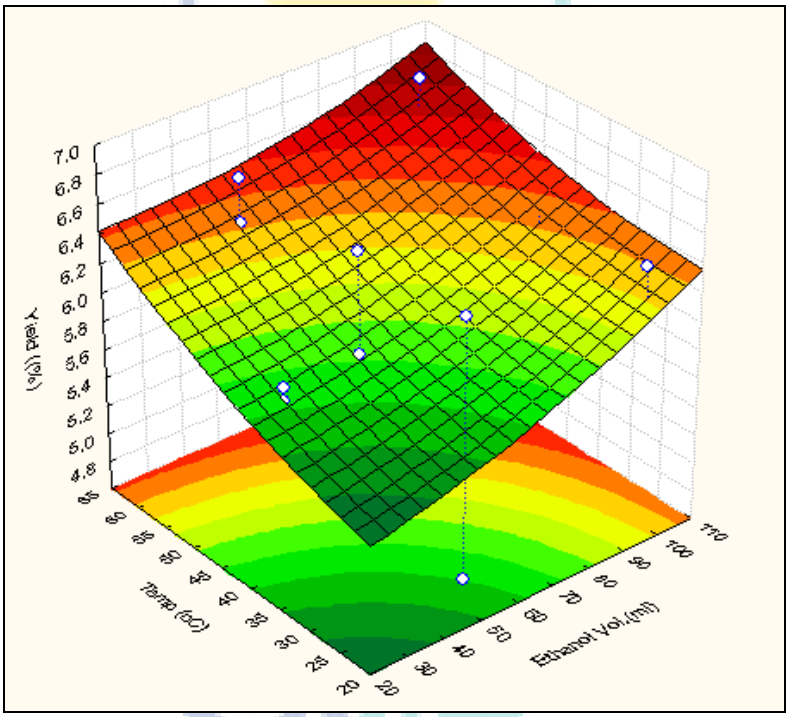


Figure 4.5 Effect of temperature and ethanol volume on the oil yield

4.3.2.2 Effect of time and ethanol volume on the oil yield

Figure 4.6 presents the effects of extraction time and volume of ethanol to the oil yield. The more extraction time and larger volume of ethanol have the effect of higher amount of oil yield. This is due to the contact time and the concentration difference as the driving force. Hence, the mass transfer of oil from the cell to the solvent will be faster. The concentration of oil in the solvent will determine the rate

of extraction at a certain extraction time. In a saturated concentration of oil in the solvent, increasing the extraction time will not make significant effect on the oil yield. (Suslick, 1989).

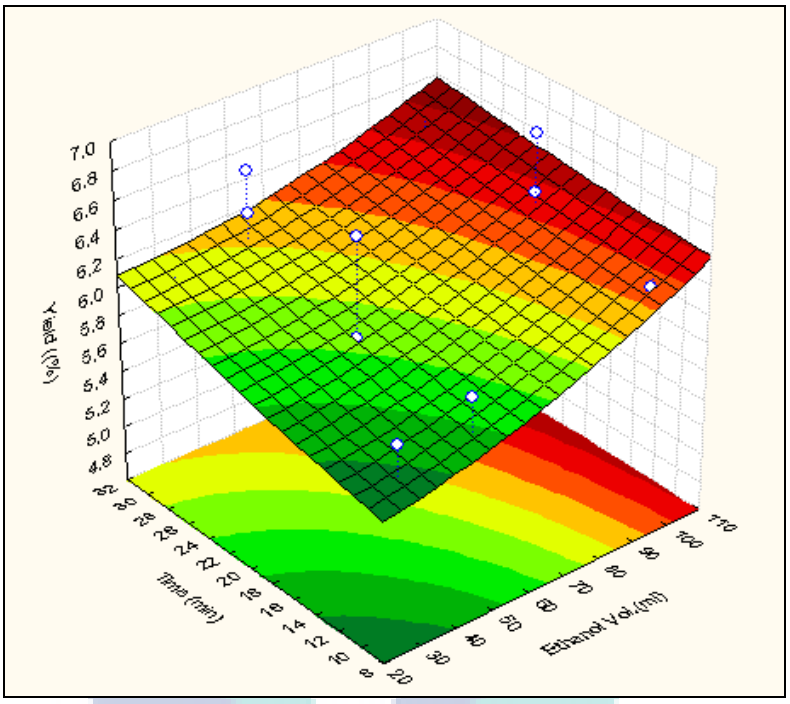


Figure 4.6 Effect of ethanol volume and time on the oil yield

4.3.2.3 Effect of temperature and time on oil yield

Figure 4.7 shows the effect of temperature and extraction time on the oil yield. This figure shows that the effect of temperature and time were significant to ethanol solvent.

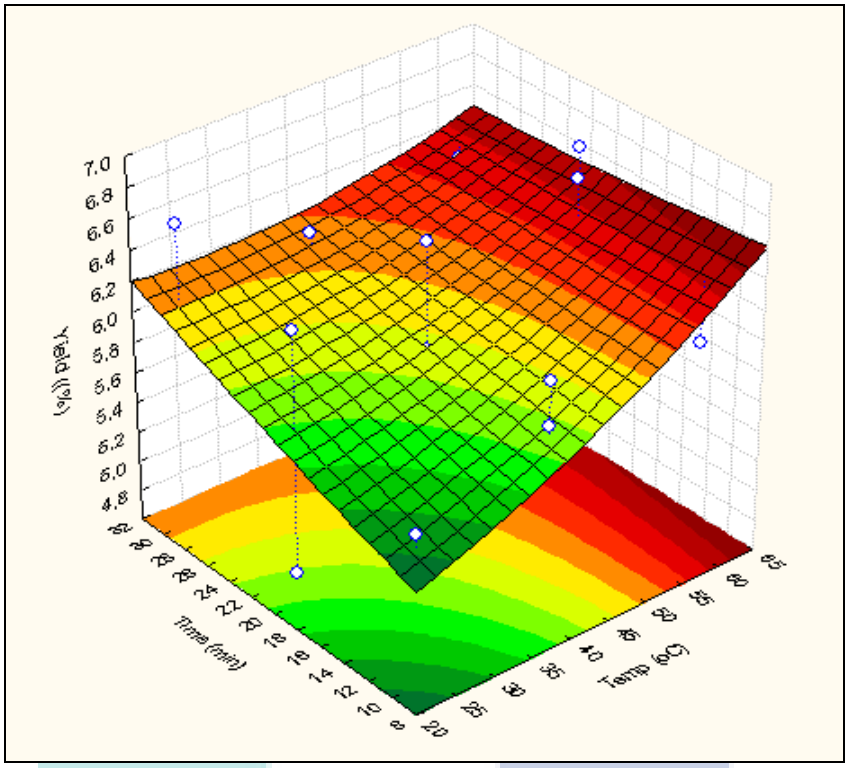


Figure 4.7 Effect of temperature and time on the oil yield

The result from the soxhlet extraction reveals that the ethanol purity influenced the algae oil quality (as indicated by FFA level and saponification number). The better result was gained when the ethanol purity was 70%. Meanwhile, the circulation time also influences the quality of oil yield. The amount of optimum time was 200 minutes or 3.3 hours. Ethanol selectivity increased gradually as its purity raised. To some extent, the increasing of purity decreased the selectivity. While, the use of ultrasonic in extraction reduced the time and temperature significantly; it needed only 51.6 minutes in 69.62^{o}C to achieve optimum oil yield. The GCMS test of algae oil component indicated that there was no significant difference between both methods of extraction in fatty acid component (Table 4.6).

The optimum time and temperature, proposed which is shown in Table 4.2, are at 51.60 minutes and 69.62^{O}C, respectively. So, comparing to the soxhlet extraction, ultrasonic extraction has shorter time and needs lower temperature. As presented in Table 4.3 it is clear that with ethanol solvent, combination of temperature and time was the most significant in extraction the oil comparing with other combinations. This can be seen from *p* value which is relatively small i.e. 0.646014. This data shows that the high level of ethanol solvent (96%) is not good to be used as the solvent in algae oil extraction.

Table 4.2 Critical values of variables using ethanol

Factor	**Observed Minimum**	**Critical Values**	**Observed Maximum**
Ethanol	30	66.99	100
Temp (C)	23	69.62	60
Time (min)	10	51.60	30

Table 4.3 Effect estimate of parameters using ethanol solvent

	Effect	**Std. Err**	**T**	**P**
Mean/Interaction	**6.155625**	**0.216681**	**28.40876**	**0.000001**
(1)Ethanol (L)	0.501503	0.639859	0.78377	0.468658
Ethanol (Q)	-0.053537	0.489603	-0.10935	0.917179
(2)Temp (L)	0.565093	0.470467	1.20113	0.283489
Temp (Q)	-0.075344	0.384319	-0.19605	0.52292
(3)Time (L)	0.266211	0.487488	0.54609	0.608478
Time (Q)	-0.018128	0.370657	-0.04891	0.962886
1L by 2L	-0.138701	0.755072	-0.18369	0.861472
1L by 3L	-0.096717	0.632634	-0.15288	0.884471
2L by 3L	-0.322246	0.659961	-0.48828	0.646014

4.4 Screening of Variable on Ultrasonic Extraction-Assisted

Using Box Behnken Design of Statistica Software, the screening was done toward the finding most influential factors in extraction and *in-situ* transesterification process. This process was to get the dominant factor among many factors in this research. Based on the data analysis, the result is as shown in the Pareto chart (Figure 4.8).

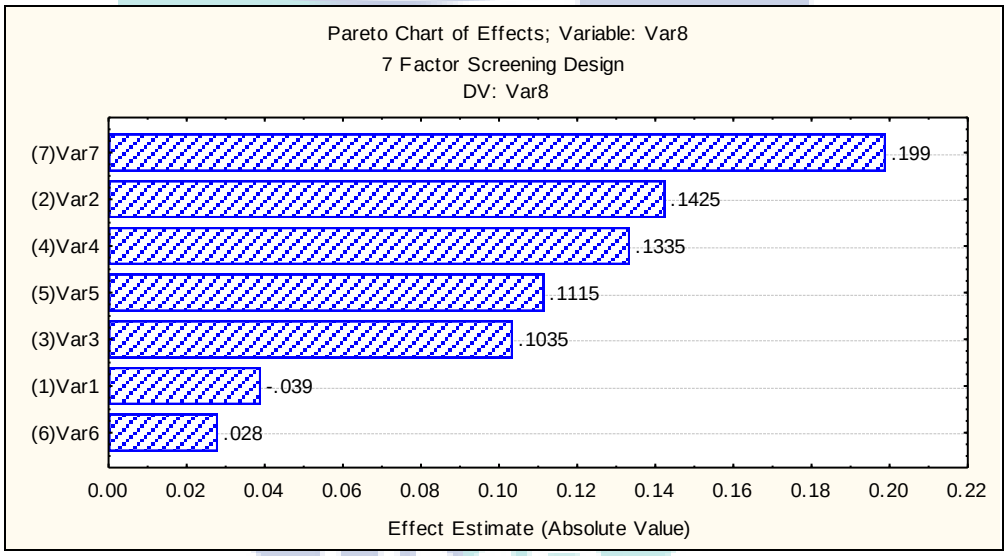


Figure 4.8 Figure Pareto Chart of Effect

As can be seen in Figure 4.8 above and based on Table 3.2, it can be understood that the most influential effects as in order are (1)Variable 7 (Ultrasonic Pulse), (2) Variable 2 (Ultrasonic Time), (3) Variable 4 (Ultrasonic Power), (4) Variable 5 (Type of Solvent), (5)Variable 3 (Solvent Purity), (6)Variable 1 (Ratio), and (7) Variable 6 (Mixing), respectively. Furthermore, three of the most influential factors were selected. These are (1).Ultrasonic pulse, (2).Ultrasonic power and (3).Ultrasonic time. These three factors were then combined in this research to identify the interaction between factors and the optimum impact toward the oil yield.

4.5 Interaction of Ultrasonic Variable on Oil Yield

The effect of interaction amongst variables in the process of extraction was studied using Box Behnken Design to get the optimum condition of extraction process and transesterification.

4.5.1 Effect of Power and Pulse on the Oil Yield

The effect of ultrasonic power dan ultrasonic pulse on the oil yield resulted in the extraction process can is show in Figure 4.9a and the *in situ* process can be seen in Figure 4.9b. In the extraction process (Figure 4.9a), on the effect of ultrasonic power, it is clear that the increase of ultrasonic power drastically increase the amount of oil yield. This result was also shown by some other researchers (Zhang. *et al*, 2007; Yaqin, *et al*, 2007). Meanwhile, in the ultrasonic pulse, the increasing of ultrasonic pulse increases the yield, but at some points the increasing of the pulse will decrease the percentage of yield. The optimum result was gained when the pulse was 75% and yield of 0.1%.

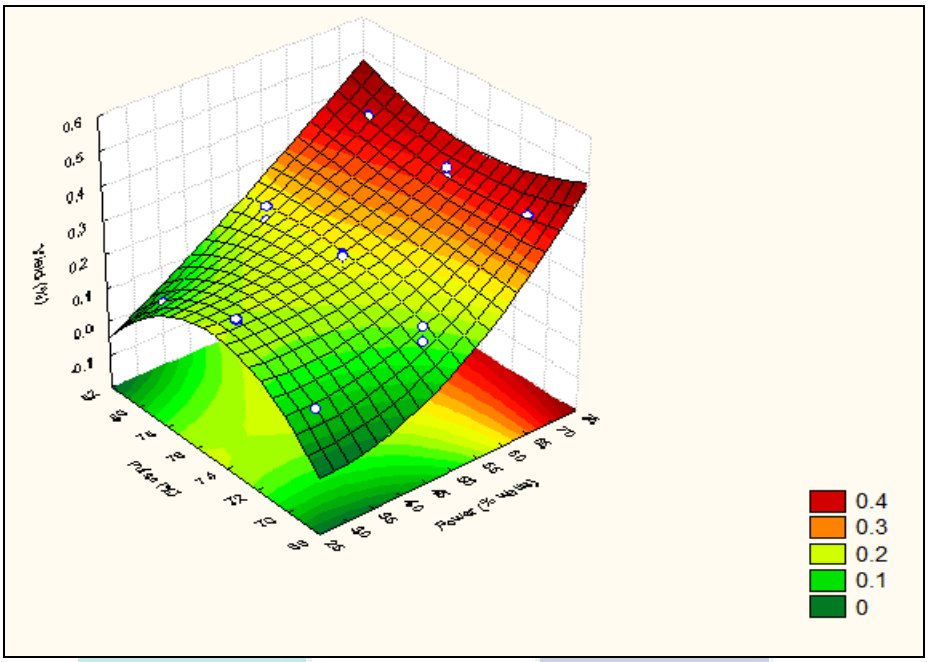


Figure. 4.9a Effect of ultrasonic power and pulse on oil yield (extraction process)

In the *in situ* process (Figure 4.9b), seen the increasing of ultrasonic power and pulse will increase the yield gained. Both variables tend to approach their optimum points. The increase of ultrasonic power results in significant yield comparing to ultrasonic pulse.

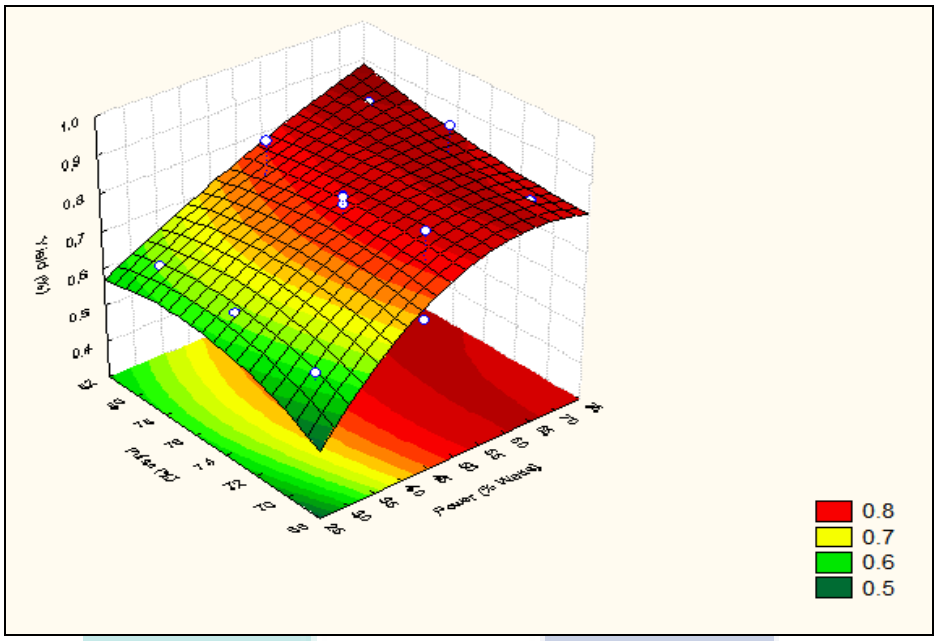


Figure 4.9b Effect of ultrasonic power and pulse on FAME yield (*in situ* process)

In the graph of ultrasonic power influence on oil yield, there is a significant increase in the early treatment and it tends to be stagnant (the stable graph) when the power is at 70% capacity (210 W). It also happens to the graph of ultrasonic pulse to the yield, which tends to be stable when the power is at 75% capacity. The result indicates that the conversion of yield increases linearly with the rise of ultrasonic power and ultrasonic pulse and at a faster rate with power than pulse.

Overall, the study on the effect of interaction between ultrasonic power and ultrasonic pulse on both extractions process and *in situ* revealed that ultrasonic power tended to give significant increasethan ultrasonic pulse, while in the ultrasonic pulse; the graph was optimum at the pulse of 75%. This result is in line with the study by Li *et al* (2004) that the increase in power and pulse (wave intensity) lead to increase in the yield.

4.5.2 Effect of Power and Time on the Yield

Figure 4.10 a-b, shows the correlation between power and time with the yield. For the extraction process (Figure 4.10a) it was observed that the effect of ultrasonic power toward the yield is more significant than ultrasonic time.

Similar case also happens in the oil extraction from flaxseed in the similar frequency (20 KHz) and similar solvent (methanol) as there was not significant increase (Zhang, 2007). The significant increase of oil yield occured in the first 30 minutes. The same thing was also shown by hepiridinextracion in Penggan (Citrus reticulata) peel. The different result was only shown by different power (Yaqin, *et al*, 2007). This is because the strength of the power of ultrasonic is more able to break the microalgae cell wall and yield the oil than the contact duration between cell and the solvent. Therefore the increasing of ultrasonic power is more significant than ultrasonic time (Figure 4.10a)

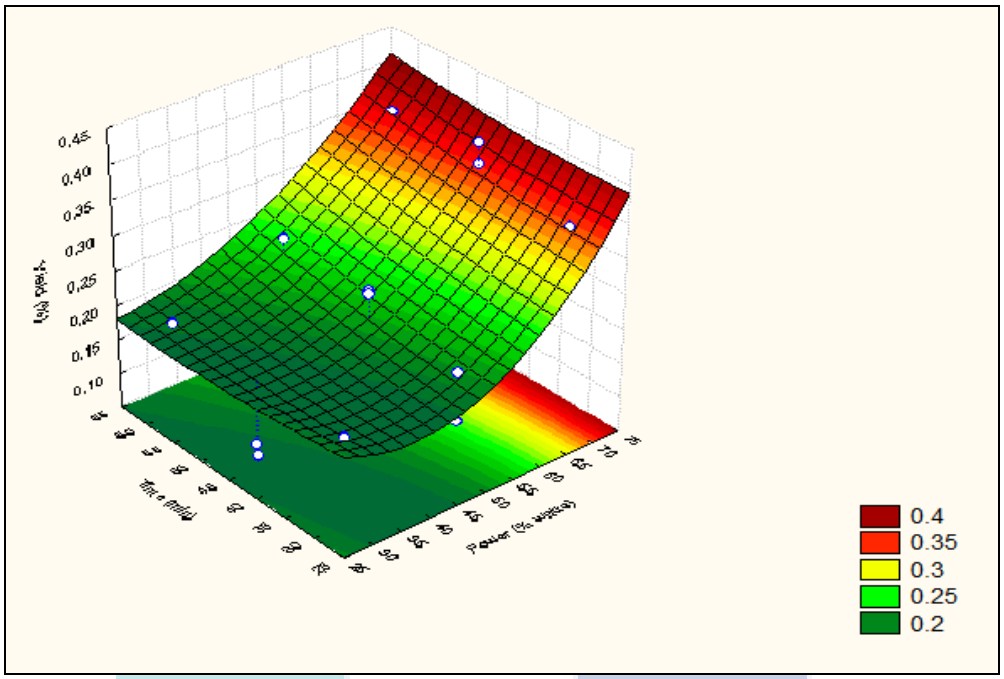


Figure 4.10a Effect of ultrasonic power and time on oil yield (extraction process)

Different result was shown in the *in situ* process (Figure 4.10b) in which the increase of both variables did not give a significant effect on the yield, but the correlation between the two tends to show optimizing graph of the *in situ* process that had been conducted.

In the effect of duration of extraction time, the graph tended to be optimum, in which the increase of time will increase the oil yield in the beginning of the process, but then it tended to decrease at particular point. The yield tends to be increasingatminute 30 up to 60 and decreasing at minute 60 up to 85. This is similar to the previous researches (Wu, *et al*, 2001; Ma *et al*., 2006), which reported that the yield reached the optimum after 40 minutes (Georgogianni *et al*, 2008). Meanwhile, Widjaja, *et al* (2009) had reported that the optimum point was reached at 15x3 minutes and it tends to decrease after 30x3 minutes.

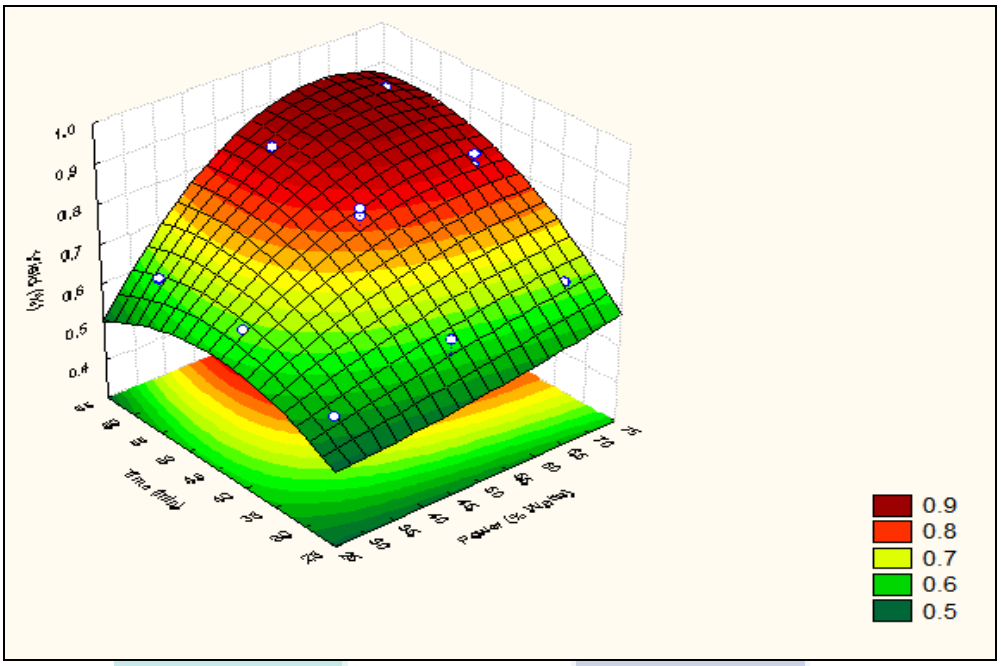


Fig 4.10b Effect of ultrasonic power and time on FAME yield (*in situ* process)

4.5.3 Effect of VariableTime and Pulse on Yield

Figure 4.11a shows the interaction graph between pulse and time in the extraction time, while Figure 4.11b shows the interaction between pulse and time for *in situ* process. In the extraction process (Figure 4.11a) it is seen that the increasing of pulse results in the increasing of the yield up to particular limit (75% pulse). After this, the increasing of pulse tends to decrease the oil yield. It is different with the result shown by variable time, in which the increasing of time will give the increasing of yield.

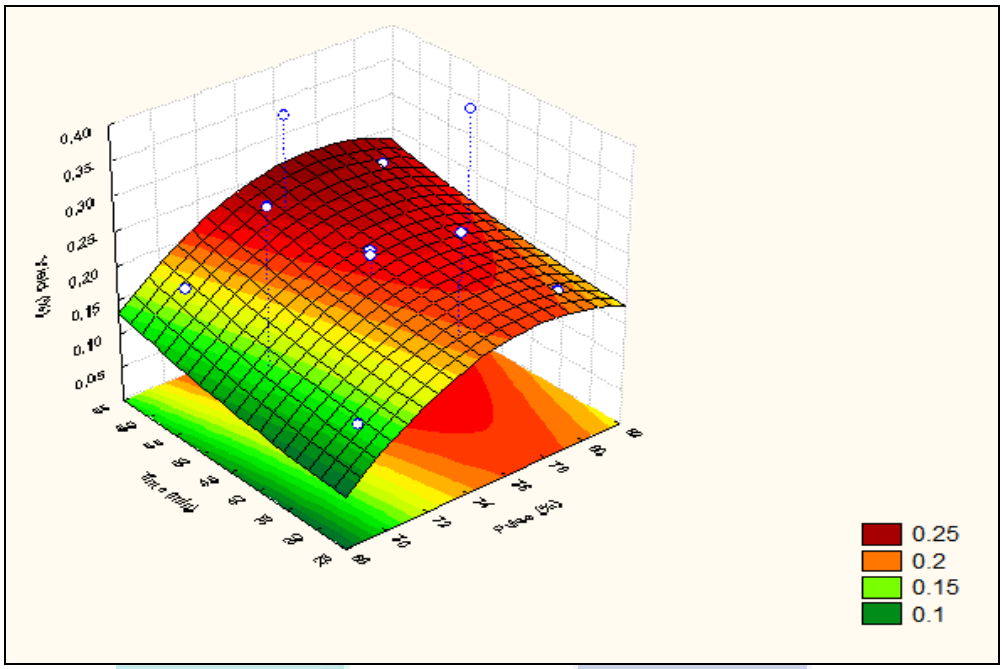


Figure 4.11a Effect of ultrasonic time and pulse on oil yield (extraction process)

In the *in situ* process (Figure 4.11b), it is clear that the influence of ultrasonic time is more significant than pulse. In this case, it is also clear from the graph that pulse addition tends to reduce the yield. In combination with pulse, it was shown that the more the time used, the more yield will be gained. This condition was slightly different as time length was combined with Ultrasonic power. This shows that the correlation between time and power gives more significant result than correlation between time and pulse.

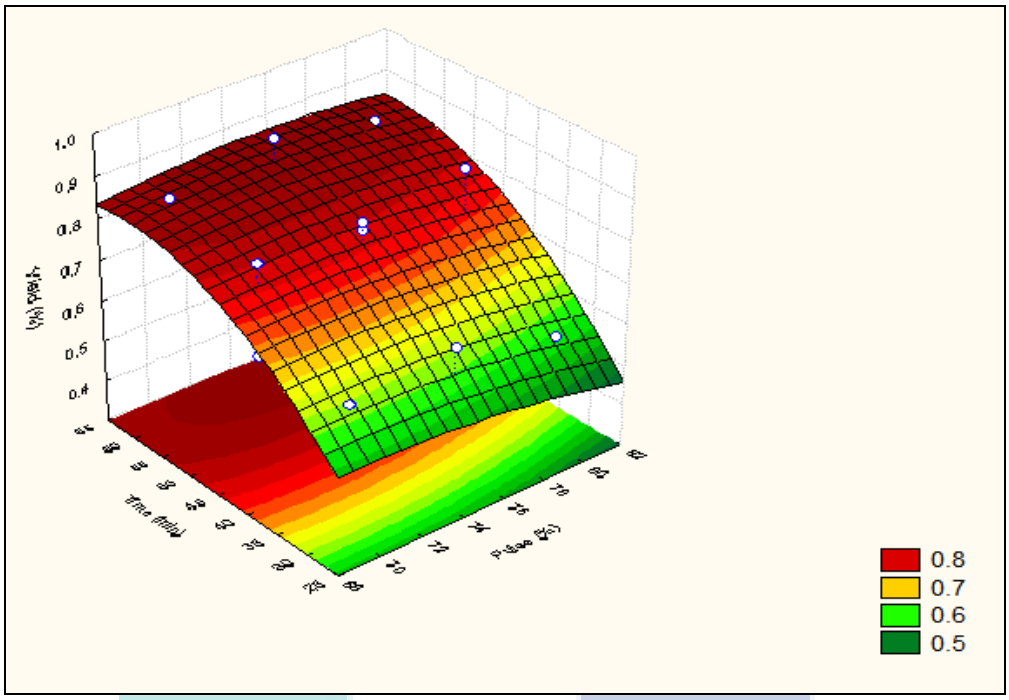


Figure 4.11b Effect of ultrasonic time and pulse on FAME yield (*in situ* process)

From all interactions in the extraction process and *in situ* transesterification (Figure 4.9-11) there is similarity in the effect of each variable. The increase of ultrasonic power and ultrasonic time tends to increase the yield as well. Both variables are more dominant in the extraction process and *in situ* ultrasonic transesterification toward the yield resulted. Figure 4.12 shows the ultrasonic power in the optimization of microalgae oil extraction is the most dominant variable comparing to ultrasonic time and ultrasonic pulse, as *p* value was 9.0286. While in Ultrasonic power and ultrasonic time as the most dominant variable comparing to ultrasonic pulse as *p* values were 4.126066 and 4.057866 (Figure 4.13).

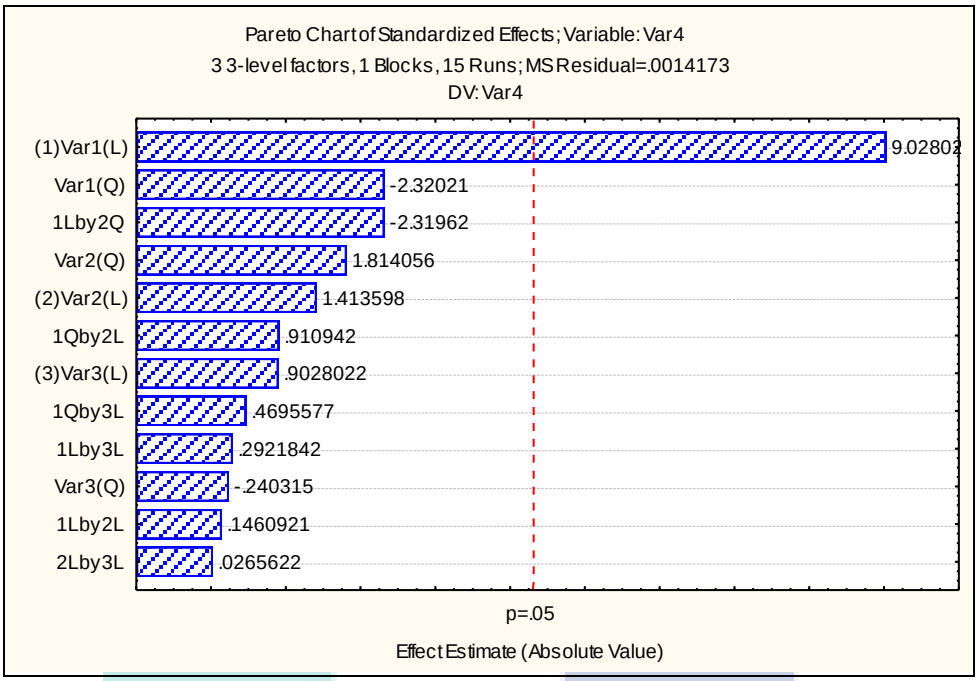


Figure 4.12 Pareto Chart of Effect on Extraction process

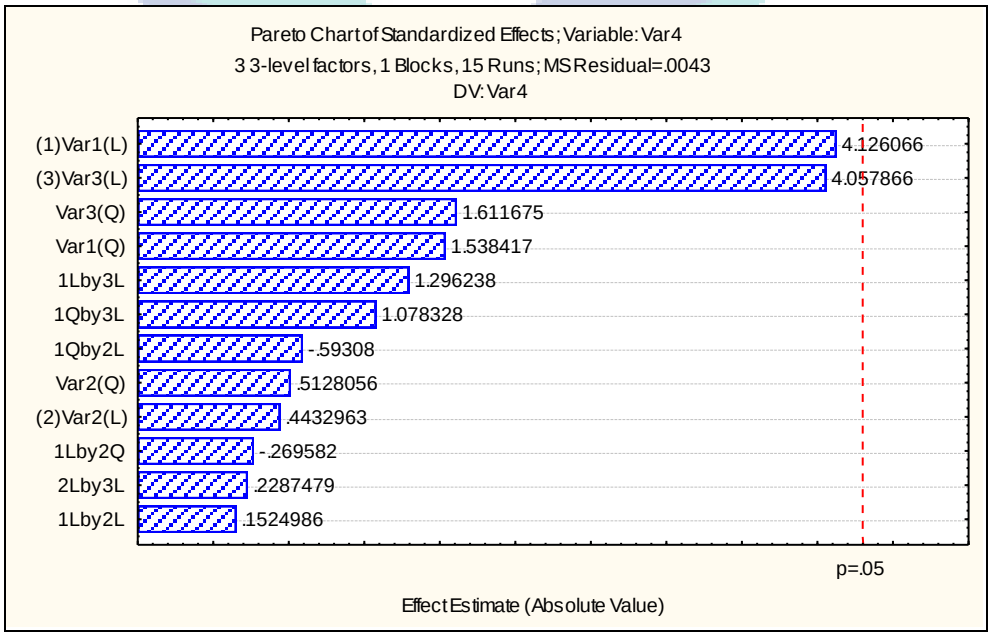


Figure 4.13 Pareto Chart of Effect on *In situ*Transestrification Process

In the event where there is an increasing ultrasonic power caused by the large amplitude ultrasonic wave traveled though a liquid medium, more bubble were created and collapsed. Since the temperature and pressure were very high inside the bubbles and the collapse of bubbles occurred over very short time, the violent shock wave and high speed jet were generated which could enhance the penetration of the solvent into the cell and accelerate the intercellular product release into the solvent by disrupting the cell walls. Moreover, the violent shock and high speed jet might have caused the molecules to mix better enhancing the mass transfer. Due to the presence of the hard cell walls which are not so permeable, the large increase in ultrasonic power result in moderate rise in yield. The same was reported by Li *et al* (2004) and Sivakumar *et al* (2007) in their studies that the increasing of ultrasonic powerwould increase the yield gained.

In relation with the effect of time length (duration) of the extraction of all ultrasonic methods, it was revealed that it led to the increasing of yield (Shan, at all, 2008). In this experiment, it was observed that the yield increased occurred in the first 30 minutes. The more the time and the longer the contact between the solvent and microalgae, the more oil will be produced. Furthermore, the ultrasonic wave, which annoys the cell wall of microalgae, influenced the mass transfer from the body of microalgae. This is in line with the result of study done by some researchers such as Hemwimol *et al*, (2006) and Balachandran *et al*, (2006).

Based on the graph, it is clear that the increasing of pulse shows different effects, but it shares similarity as well, and at some particular points the ultrasonic pulse will increase the yield (Li, *et al*, 2004) and reaches the optimum (Figure 4.9a, 4.9b, 11a and 11b).

In pulse operation, the ultrasonic energy is turned on and off at a rate which may vary from once every several seconds to several hundred times per second.

The percentage of time that the ultrasonic energy is on may also be changed to produce varied results At slower pulse rates, more rapid degassing of liquids occurs as coalescing bubbles of air are given an opportunity to rise to the surface of the liquid during the time the ultrasonic energy is off. At more rapid pulse rates the extraction process may be enhanced as repeated high energy "bursts" of ultrasonic energy occurred each time the energy source is turned on.

The increase of oil yield can be explained by cavitation effect caused by the use of high intensity ultrasound. As large amplitude ultrasound wave travel through a mass medium, they cause compression and shearing of solvent molecules resulting in localized change in density and elastic modulus (Price, et al, 1995) As a consequence, the initially sinusoidal compression and shear waves will at a finite distance from the ultrasonic transducer be distorted into shock waves. The abrupt decrease in pressure at the edge of the saw tooth shaped ultrasonic wave in the negative pressure cycle generates small bubbles. These bubbles collapse in the positive pressure cycle and produce turbulent flow conditions associated with high pressures and temperatures (Mason, 1990; Mason, *et al*. 1996). Since formation and collapse of bubbles occurs over very short periods of time, typically a few microseconds (Hardcastle *et al*., 2000), heat transfer from cavitation bubbles to the medium is small causing only gradual temperature increases in the medium. Therefore, decreases in solvent viscosity are small and are most likely not the principal cause of the yield increases. Rather, at increasing amplitudes, cavitation bubble collapse is more violent since the resonant bubble size is proportional to the amplitude of the ultrasonic wave (Suslick, *et al*, 1987; Suslick and Price, 1999). Bubble collapse in the vicinity of plant membranes may cause strong shear forces to be exerted that can cause micro fractures to be formed in plant tissues (Vinatoru, 2001; Vinatoru *et al*., 1997).

The classical techniques for the solvent extraction of materials from vegetable sources are based upon the correct choice of solvent coupled with the use of heat and/or agitation. Solvent extraction of organic compounds contained within the bodies of plants and seeds are significantly improved by the use of power ultrasound. The mechanical effects of ultrasound provide a greater solvent penetration into cellular materials and improve mass transfer due to the effects of micro streaming (Gogate, *et al*, 2009). This is combined with an additional benefit for the use of ultrasound in extractive processes: the disruption of biological cell walls to facilitate the release of contents. Overall, ultrasound-assisted extraction is now recognized as an efficient extraction technique that dramatically cuts down working times, increasing yields and often the quality of the extract.

4.6 ANOVA for Response

The results of the responses for oil yield (Y) found from experimental work and estimated by the Box-Behnken Design is presented in Tables 4.4 and 4.5. An appropriate procedure for analyzing a Box-Behnken Design is based on analysis of variance, which is summarized in the ANOVA table, as given in Table 4.4, to identify significance of effects or interaction of factors on a response.

Tabel 4.4 ANOVA for response surface quadratic model for Extraction

Factor	Effect Estimates; Var.:Var4; R-sqr=.97977; Adj:.85837 (Spreadsheet1) 3 3-level factors, 1 Blocks, 15 Runs; MS Residual=.0014173 DV: Var4									
	Effect	Std.Err.	t(2)	p	-95.% Cnf.Limt	+95.% Cnf.Limt	Coeff.	Std.Err. Coeff.	-95.% Cnf.Limt	+95.% Cnf.Limt
Mean/Interc.	0.224083	0.010868	20.61884	0.002344	0.177323	0.270844	0.224083	0.010868	0.177323	0.270844
(1)Var1 (L)	0.253333	0.028061	9.02802	0.012048	0.132598	0.374069	0.126667	0.014030	0.066299	0.187035
Var1 (Q)	-0.045458	0.019592	-2.32021	0.146115	-0.129758	0.038841	-0.022729	0.009796	-0.064879	0.019420
(2)Var2 (L)	0.039667	0.028061	1.41360	0.293047	-0.081069	0.160402	0.019833	0.014030	-0.040535	0.080201
Var2 (Q)	0.035542	0.019592	1.81406	0.211340	-0.048758	0.119841	0.017771	0.009796	-0.024379	0.059920
(3)Var3 (L)	0.025333	0.028061	0.90280	0.461917	-0.095402	0.146069	0.012667	0.014030	-0.047701	0.073035
Var3 (Q)	-0.004708	0.019592	-0.24031	0.832473	-0.089008	0.079591	-0.002354	0.009796	-0.044504	0.039795
1L by 2L	0.005500	0.037647	0.14609	0.897244	-0.156484	0.167484	0.002750	0.018824	-0.078242	0.083742
1L by 2Q	-0.061750	0.026621	-2.31962	0.146173	-0.176290	0.052790	-0.030875	0.013310	-0.088145	0.026395
1Q by 2L	0.024250	0.026621	0.91094	0.458484	-0.090290	0.138790	0.012125	0.013310	-0.045145	0.069395
1L by 3L	0.011000	0.037647	0.29218	0.797668	-0.150984	0.172984	0.005500	0.018824	-0.075492	0.086492
1Q by 3L	0.012500	0.026621	0.46956	0.684888	-0.102040	0.127040	0.006250	0.013310	-0.051020	0.063520
2L by 3L	0.001000	0.037647	0.02656	0.981221	-0.160984	0.162984	0.000500	0.018824	-0.080492	0.081492

Std. Err : Standard Error

Cnf. Limt : Confident Limit

Coeff : Coefficient

Std. Err. Coeff : Standard Error Coefficient

Tabel 4.5 ANOVA for response surface quadratic model for *In situ*

Factor	Effect Estimates; Var.:Var4; R-sqr=.98743; Adj:.91198 (Spreadsheet1) 3 3-level factors, 1 Blocks, 15 Runs; MS Residual=.0013 DV: Var4								
	Effect	Std.Err.	t(2)	p	-95.% Cnf.Limt	+95.% Cnf.Limt	Coeff.	Std.Err. Coeff.	-95.% Cnf.Limt
Mean/Interc.	0.710000	0.010408	68.21459	0.000215	0.665217	0.754783	0.710000	0.010408	0.665217
(1)Var1 (L)	0.208333	0.026874	7.75217	0.016236	0.092703	0.323964	0.104167	0.013437	0.046352
Var1 (Q)	0.052500	0.018764	2.79793	0.107527	-0.028234	0.133234	0.026250	0.009382	-0.014117
(2)Var2 (L)	0.011667	0.026874	0.43412	0.706545	-0.103964	0.127297	0.005833	0.013437	-0.051982
Var2 (Q)	0.012500	0.018764	0.66617	0.573857	-0.068234	0.093234	0.006250	0.009382	-0.034117
(3)Var3 (L)	0.195000	0.026874	7.25603	0.018469	0.079370	0.310630	0.097500	0.013437	0.039685
Var3 (Q)	0.055000	0.018764	2.93116	0.099348	-0.025734	0.135734	0.027500	0.009382	-0.012867
1L by 2L	0.000000	0.036056	0.00000	1.000000	-0.155134	0.155134	0.000000	0.018028	-0.077567
1L by 2Q	-0.017500	0.025495	-0.68641	0.563352	-0.127197	0.092197	-0.008750	0.012748	-0.063598
1Q by 2L	-0.027500	0.025495	-1.07864	0.393550	-0.137197	0.082197	-0.013750	0.012748	-0.068598
1L by 3L	0.085000	0.036056	2.35748	0.142463	-0.070134	0.240134	0.042500	0.018028	-0.035067
1Q by 3L	0.045000	0.025495	1.76505	0.219601	-0.064697	0.154697	0.022500	0.012748	-0.032348
2L by 3L	0.025000	0.036056	0.69338	0.559775	-0.130134	0.180134	0.012500	0.018028	-0.065067

Std. Err : Standard Error

Cnf. Limt : Confident Limit

Coeff : Coefficient

Std. Err. Coeff : Standard Error Coefficient

From the ANOVA for response surface quadratic model for surface area, the model *p*-values of 0.002344 (extraction) and 0.000215 (*in situ*) imply that the models are significant.

Figures 4.14 and 4.15 shows the experimental values versus predicted values using the model equation developed by STATISTICA Software version 6.0. A line of unit slope, the line of perfect fit with points corresponding to zero error between experimental and predicted values is also shown in Figures 4.14. The coefficient of correlation (R^2) is 0.97977 (extraction) and 0.98743 (*in situ*). The results in Figure 4.14 and 4.15 demonstrate that the regression model equation provides an accurate description of the experimental data, indicating that it has successfully captured the correlation between the three parameters to oil and biodiesel yield.

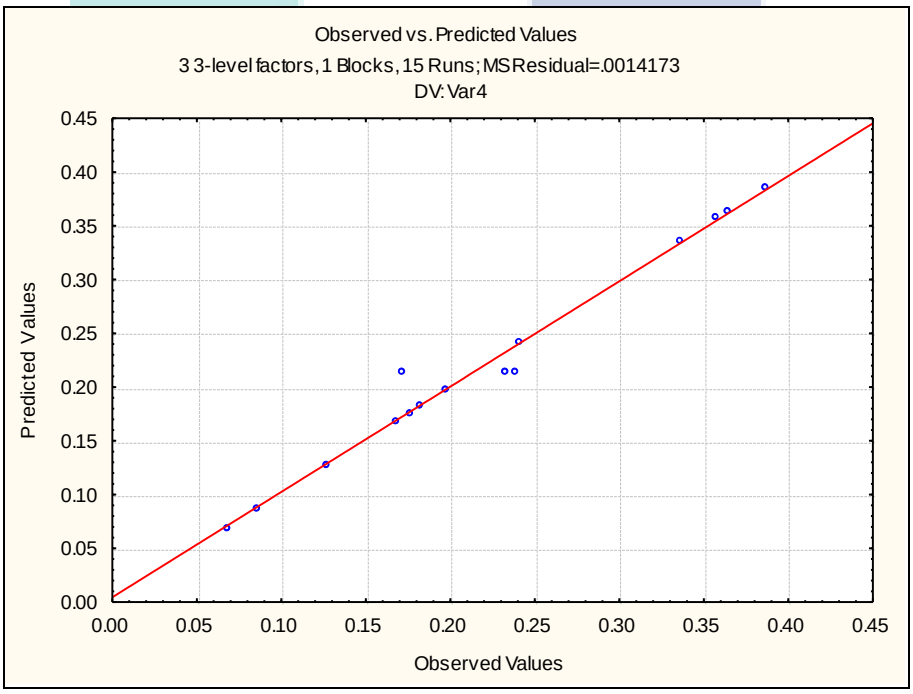


Figure. 4.14 A comparative plot between experimental and predicted oil yield for extraction

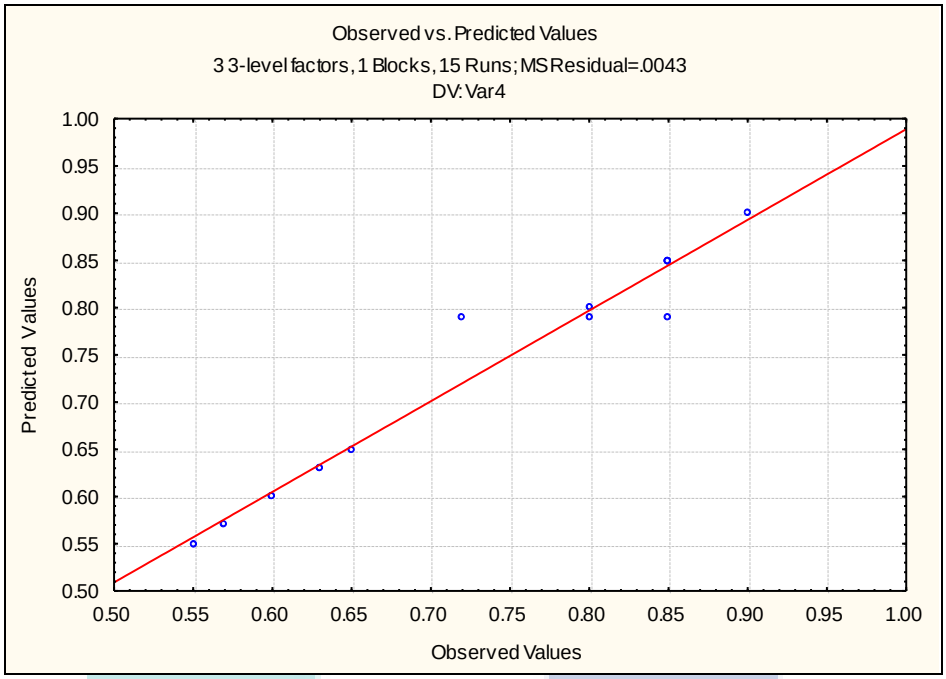


Figure. 4.15 A comparative plot between experimental and predicted oil yield for *in situ*

4.7 Chemical Component of Microalgae Oil

Figure 4.16 shows the top layer consists of the mixture of lipid-hexane and the bottom layer consist of water and methanol. The top layer was taken and then separated by heating to remove the hexane, replace the wax ester, and other impurities to get clean microalgae oil (Figure 4.17).

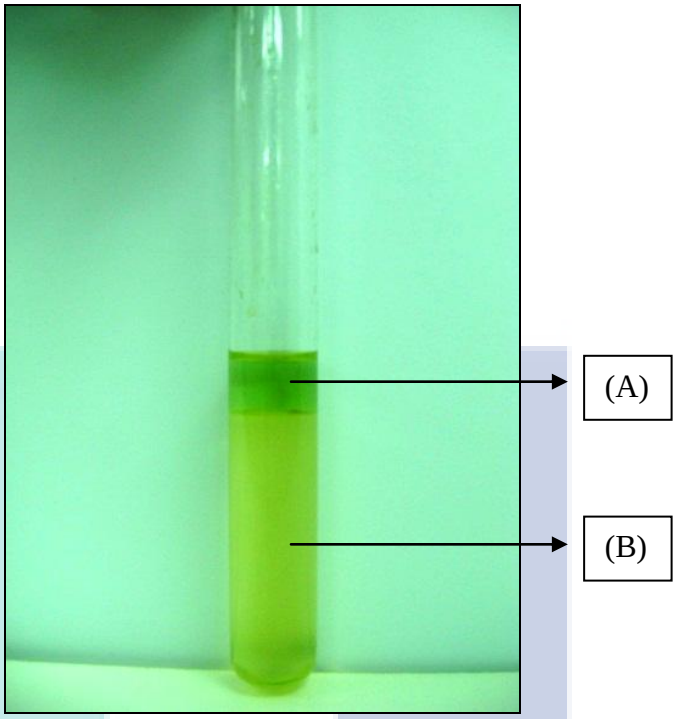


Figure 4.16 Fractions of lipid-hexane (A) and water-methanol (B)

The major fatty acid compositions of the tested microalgae were determined using GCMS Analysis (Tabel 4.6). These results are consistent with the reported by Yon, *et al*, (2010) In the three tested microalgae, oleic acid (C18:1) and linoleic acid (C18:2) were commonly dominant. The FA content of microalgae depends on the strains and the culturing condition. The composition of medium, the aeration rate, light intensity, duration of the photoperiod, temperature and the age of culture are some culturing condition which influence the FA content (Medina, at al., 1998).

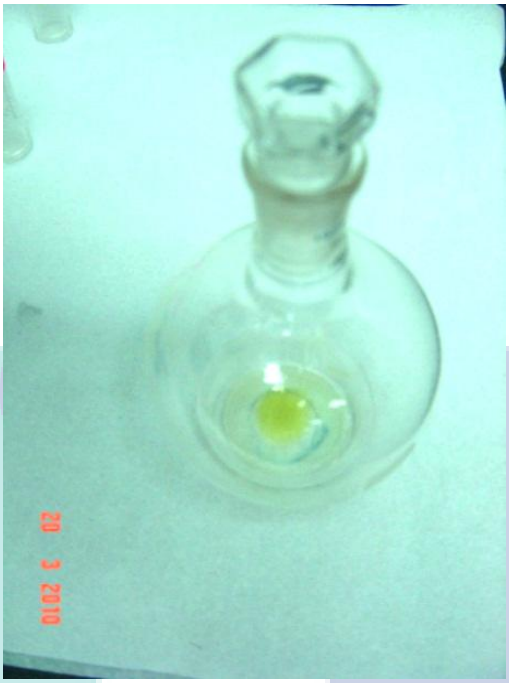


Figure 4.17 Microalgae Oil after purification

Based on the test on free fatty acid purity and saponification number, it is known that the FFA level was 19.6% and the saponification number is 114.233 KOH/1g oil. The content of FFA is categorized high for biodiesel production process. The content of vegetable oil which is usually used as the raw material of biodiesel is ranging approximately 2%-5%. The high level of FFA is caused by some factors. Besides the type of the source (microalgae), it can also be caused by incorrected process. Here, the possible cause is overheating and types of microalgae sample used.

Tabel 4.6 Comparison of Fatty acid composition of *Nannochloropsis sp.*via ultrasonic and Soxhlet methode

No	Fatty Acid	Name	Molecule Mass (g/mol) (MMfa)	Via Ultrasonic		Via Soxhlet	
				% in Sample	Molecule mass Contribution (g/mol) (MMc)	% in Sample	Molecule mass Contribution (g/mol) (MMc)
1	C 12	Dedecanoic acid	200.30	16.300	32.649	16.240	32.528
2	C14	Tetradecanoic acid	288.40	11.143	32.136	10.965	31.623
3	C16:0	Hexadecanic acid	256.42	12.517	32.096	12.250	31.411
4	C18:0	Octadecanoic acid	284.40	5.626	16.000	5.565	15.826
5	C18:1	Octadecenoic acid	282.46	43.492	122.847	43.556	123.028
6	C18:2	Octadecadinoic acid	280.45	5.858	16.428	5.755	16.139
Average molecular mass of constituent fatty acid (MMFA)					252.156		250.555

Data Table 4.6 (ultrasonic data) was used to determine the average molecular mass of the *Nannochloropsis sp* oil. Since the microalgae oil (assumed to be primarily composed of triglycerides) consists of different fatty acids, their respective contributions to the overall molecular mass of the microalgae lipid was used (as seen in the 'MMc' column in Table 4.6) to estimate the mean molecular mass of the constituent lipid fatty acids (MM_{fa}). With the formation of the triglyceride molecule facilitated by the combination of fatty acid molecules and a molecule of glycerol with the condensation of three molecules of water, the average molecular mass of the microalgae oil (MM oil) can be calculated using Eq. 1

$$MM_{oil} = [3MM_{fa}+MM_{glycerol}] - 3MM_{water} \quad (1)$$

where, $MM_{glycerol}$ and MM_{water} represent the molecular masses of glycerol and water, respectively. The average molecular weight of the *Nannochloropsis sp.* lipid was calculated to be 794.5 gram/mol.

For the industrial scale, the microalgae productivity as fuel is promising. In the culturing system open pond with volume of 200.000 liter (77m x 14m x 0.2m) the microalgae powder resulted is about 1.500 kg alga/week. The based lipid taken is 753.3 kg of CAO (crude algae oil) and Triglyceride in CAO is of 93.45% following the chemical/reaction equation bellow,

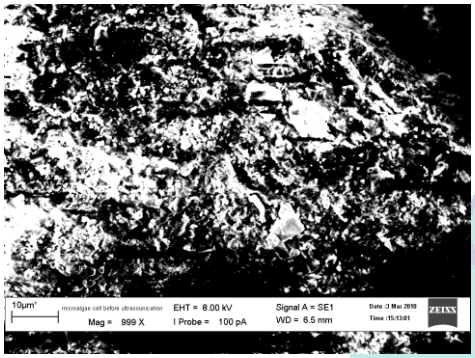
$$1[H2O+FFA+TG] + 3\ MeOH \rightarrow 3\ Biodiesel + 1\ Glycerin \quad (2)$$

So, the volume of product of biodiesel microalgae *Nannochloropsis sp.* per week is 825.16 L/pond/week.

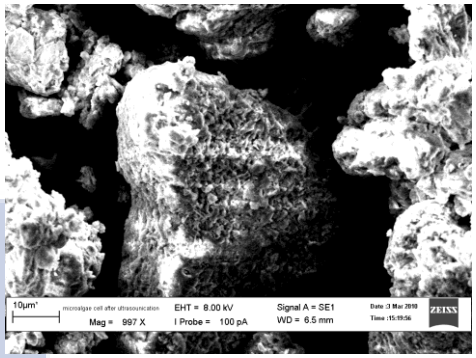
4.8 Effect of Ultrasonic on the physical structure

The Scanning Electron Microscopy (SEM) images of the microalgae powder at a various magnification factor (307X and 1000X) are shown in Figure. 4.18

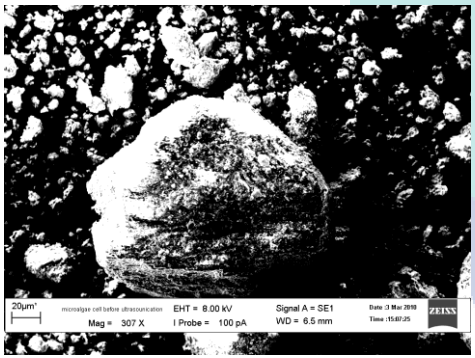
On the left side (Figures 4.18A and 4.18B) was the visualization of powder before ultrasonication treatment. It is observed that the grain of microalgae is still complete. Figure 4.18C and 4.18D shows the structure of microalgae cell after 30 minutes being exposed to ultrasonic. It can be seen that the cell wall was broken. The break allows the penetration of the solvent to take the oil from the microalgae cell.



A (1K)

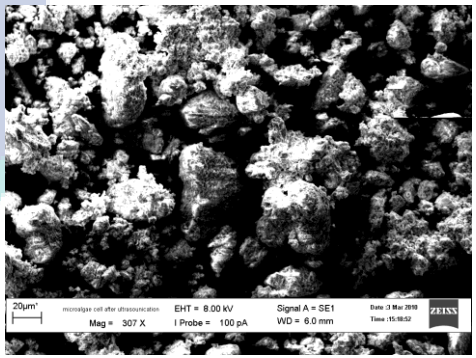


C (1 K)



B (307 x)

Before Ultrasonication



D (307 x)

After Ultrasonication

Figure 4.18 Visualization of powder before ultrasonication treatment

CHAPTER 5
CONCLUSION AND RECOMMENDATIONS

5.1 Conclusions

This research was done to investigate the effect of ultrasonic use in the extraction process and *in situ* transesterification process of microalgae into biodiesel. This research consists of two steps, 1) screening and 2) optimizing. Seven factors were used in the screening stage namely microalgae-solvent ratio, ultrasonic time, solvent concentration, ultrasonic power, type of solvent, mixing and ultrasonic pulse.

In the optimizing process, three significant factors from screening process were taken to determine the optimum condition. The Box Bhenken Design of STATISTICA version 6.0 was used in both stages to analyze the result.

The main conclusions that can be drawn from this study are summarized as follow:

1) In the screening process, the most influential factors in the ultrasonic assisted factor and *in situ* transesterification factor respectively from the most to the least are; ultrasonic pulse, ultrasonic time, ultrasonic power, type of solvent, solvent concentration, microalgae-solvent ratio and mixing.

2) The variable of ultrasonic power in the optimization process of microalgae oil extractions is the most significant variable comparing to the ultrasonic time and ultrasonic pulse (*p* value = 9.0286). Meanwhile in the *in situ* ultrasonic power and ultrasonic time are more significant than ultrasonic pulse (*p* value = 4.126066 and 4.057866, respectively).

3) From all interactions in the extraction process and *in situ* transesterification there is a similarity in the effect of each variable. The increase of ultrasonic power and ultrasonic time tends to increase the yield as well. Both variables are more significant in the extraction process and *in situ* ultrasonic transesterification toward the yield resulted.

4) The chemical composition of fatty acids from*Nannochloropisis sp* strain consists of monounsaturated and polyunsaturated Octadecenoic acid (C18:1) 43.49%,Dedecanoic acid (C12) 16.30%, Hexadecanic acid (C16:0) 12.51%, Tetradecanoic acid (C14) 11.43%, Octadecadinoic acid (C18:2) 5.85% and Octadecanoic acid (C18:0) 5.62%.

5) Oil algae characteristic have density of 0.924 g/ml, Saponification Number 114, 269 KOH/1 g oil and 19.67% FFA.

5.2 Recommendation

Recommendations are made to suggest for future work which can be performed to give better understanding and improvement on the oil extraction system. Below are some recommendations for future work:

1) The selection of the best microalgae strain as the biodiesel source, i.e. microalgae with high lipid content, is needed.

2) Further research on the process development for pilot plan scale is needed to assure the sustainability of the production process of biodiesel microalgae.

3) Further research on the use of solid (biomass) waste to give additional value for the development process of biodiesel microalgae is needed.

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APPENDIX A

LIST OF PUBLICATION

A.1. Journal

1. Wiyarno, Budi and MohdYunus, Rosli and Mel, Maizirwan (2010) *Ultrasonic Extraction Asissted (UEA) of Oil from Microalage (Nannochloropsis .sp)* International Journal of Engineering and Science Vol 1 No 3, 2010. ISSN 2086-3799.

2. Wiyarno, Budi and MohdYunus, Rosli and Mel, Maizirwan (2011) *Extraction of Algae Oil from Nannocloropsis sp.: A study of Soxhlet and Ultrasonic-Assisted Extractions.* Journal of Applied Sciences. ISSN 1812-5662 (O), 1812-5654 (P) (In Press) http://scialert.net/fulltext/?doi=jas.2011.3607.3612&org=11

A.2. International Conference

1. Wiyarno, Budi and MohdYunus, Rosli and Mel, Maizirwan (2010) Ultrasonic Extraction Asissted (UEA) of Oil from Microalage (*Nannochloropsis sp*.). UniversitasMalahayati Lampung, Indonesia

2. Wiyarno, Budi and MohdYunus, Rosli and Mel, Maizirwan (2011) *Extraction of Algae Oil from Nannocloropsis sp.: A study of Soxhlet and Ultrasonic-Assisted Extractions.* ICPEAM 2010 Universiti Teknologi Petronas, Malaysia

APPENDIX B

TAXONOMY OF NANNOCHLOROPSIS

Hibberd, D.J. (1981)

Empire	: Eukaryota
Kingdom	: Chromista
Subkingdom	: Harosa
Infrakingdom	: Heterokonta
Phylum	: Ochrophyta
Subphylum	: Phaeista
Infraphylum	: Limnista
Class	: Eustigmatophyceae
Order	: Eustigmatales
Family	: Monodopsidaceae
Genus	: Nannochloropsis

APPENDIX C

ANALYSIS OF MICROALGAE POWDER CONTENT

Sample Name : Microalgae (Nannochloropsissp)

Type of Sample : Powder

No	Analysis	Result
1	Carbohydrate	0.46%
2	Water	11.18%
3	Ash	60.02%
4	Protein	4.33%
5	Lipid	4.77%

PROKSIMAT ANALYSIS

SAMPLE: *Nannochloropsis sp.*

A. CARBOHYDRATE

No	Sample (gr)	Na2S2O3 0.1017 N (ml)	% Carbohydrate
1	2.5262	24. 70	0.46
2	2.5269	24.70	0.46

Sample 1

(ml Bl - ml smpl) ➔ (25.10 - 24.70) : 0.40 ml Na2S2O3 0.1017 N

= 0.40 x (0.1017/0.1) ml : 0.41 ml Na2S2O3 0.1017 N (look at table)

= 0.41 ml equivalent (0.41/1) x 2.9 mg powder: 1.16 mg

% Carbohydrate = ((mg powder x dilution)/(sample wight)) x 100%

= ((1.16 x (200/20)) / (2526.2)) x 100%

= 0.46 %

Sample 2

(ml Bl - ml smpl) ➔ (25.10 - 24.70) : 0.40 ml Na2S2O3 0.1017 N

= 0.40 x (0.1017/0.1) ml : 0.41 ml Na2S2O3 0.1017 N (look at table)

= 0.41 ml equivalent (0.41/1) x 2.9 mg powder: 1.16 mg

% Carbohydrate = ((mg powder x dilution)/(sample wight)) x 100%

= ((1.16 x (200/20)) / (2526.6)) x 100%

= 0.46 %

Average

= (0.46 + 0.46) / 2

= 0.46 % Carbohydrate

B. ASH

No	Sample (gr)	Ash (550^{0}C) (gr)
1	31.9283 29.9023 _ 2.0260	31.1180 29.9023 _ 1.2157

Ash Content (%)

= (1.2157/2.0260) x 100%

= 60.00%

No	Sample (gr)	Ash (550^{0}C) (gr)
2	31.2196 33.2115 _ 2.0081	34.2115 33.2115 _ 1.2057

Ash Content (%)

= (1.2057/2.0081) x 100%

= 60.02%

Average

= (60.00 + 60.02)/2

= 60.01 %

C. WATER

No	Sample (gr)	Residu (105^0C) (gr)
1	56.1648 46.3922 _ 9.7726	55.0692 46.3922 _ 8.6770

Water Content (%)

= ((sample – residu)/(sample)) x 100%

= ((9.7726-8.6770)/(9.7726)) x 100%

= 11.21%

No	Sample (gr)	Residu (105^0C) (gr)
2	53.4328 43.1972 _ 10.2356	52.2924 43.1972 _ 9.0952

Water Content (%)

= ((sample – residu)/(sample)) x 100%

= ((10.2356-9.0952)/(10.2356)) x 100%

= 11.14%

Average

= (11.21 + 11.41)/2

= 11.18 %

D. PROTEIN

No	Sample (gr)	Titration HCl 0.0978 N (ml)
1	0.5161	2.60

% Protein

= (((ml x N) HCl x AN Nitrogen x fk)/(Sample)) x 100%

= (((2.60x0.0978x14x6.25)/(516.1)) x 100%

= 4.31%

No	Sample (gr)	Titration HCl 0.0978 N (ml)
2	0.5128	2.60

% Protein

= (((ml x N) HCl x AN Nitrogen x fk)/(Sample)) x 100%

= (((2.60x0.0978x14x6.25)/(512.8)) x 100%

= 4.34%

Average

= (4.31 + 4.34)/2

= 4.33 %

E. LIPID

No	Sample (gr)	Lipid (gr)
1	8.2477	0.3982

% Lipid

= ((lipid)/(Sample)) x 100%

= ((0.3982)/(8.2477)) x 100%

= 4.83%

No	Sample (gr)	Lipid (gr)
2	8.003	0.3905

% Lipid

= ((lipid)/(Sample)) x 100%

= ((0.3905)/(8.003)) x 100%

= 4.70%

Average

= (4.83 + 4.70)/2

= 4.77 %

Table C.1 Conversion of Milliliter Thiosulphate to Milligram of starch

ml 0,1 N Thiosulphate	**mg starch/flour**	**ml 0,1 N** Thiosulphate	**mg starch/flour**
1.0	2.9	14.0	41.2
2.0	5.7	15.0	44.4
3.0	8.5	16.0	47.5
4.0	11.4	17.0	50.7
5.0	14.3	18.0	53.8
6.0	17.3	19.0	57.0
7.0	20.2	20.0	60.2
8.0	23.0	21.0	63.6
9.0	26.0	22.0	67.0
10.0	29.1	23.0	70.6
11.0	32.1	24.0	74.3
12.0	35.1	25.0	77.9
13.0	38.2		

APPENDIX D
DETERMINATION OF PERCENTAGE OF YIELD IN MICROALGAE OIL

Tabel D.1. Screening Combination and Yield

Run	A	B	C	D	E	F	G	yield (g)
1	-1	-1	-1	1	1	1	-1	0.183
2	1	-1	-1	-1	-1	1	1	0.098
3	-1	1	-1	-1	1	-1	1	0.363
4	1	1	-1	1	-1	-1	-1	0.147
5	-1	-1	1	1	-1	-1	1	0.346
6	1	-1	1	-1	1	-1	-1	0.086
7	-1	1	1	-1	-1	1	-1	0.184
8	1	1	1	1	1	1	1	0.589

Tabel D.2 Screening Parameter

Screening Parameter		1	-1
A	RasioAlga:Solvent	1:10	1:3
B	Time	45	15
C	Solvent Consetration	80%	60%
D	Ultrasonic Power	50	30
E	Solvent	Ethanol	Methanol
F	Agitate	yes	no
G	Ultrasonic Amplitude/Pulser	75%	25%

Type of solvent; (2) Ethanol, (1) Methanol, Mixing; (2) Yes, (1) No

Tabel D.3. Optimizing Combination and Oil Yield: Extraction

Run	A	B	C	Oil yield
1	30	70	45	0.068
2	70	70	45	0.357
3	30	80	45	0.086
4	70	80	45	0.386
5	30	75	30	0.176
6	70	75	30	0.336
7	30	75	60	0.182
8	70	75	60	0.364
9	50	70	30	0.127
10	50	80	30	0.198
11	50	70	60	0.168
12	50	80	60	0.241
13	50	75	45	0.171
14	50	75	45	0.239
15	50	75	45	0.233

Tabel D. 4 Optimum Combination and Yield: *In situ*Transesterification

Run	Power	Pulser	Timer	FAME Yield
1	30	70	45	0.06
2	70	70	45	0.082
3	30	80	45	0.063
4	70	80	45	0.085
5	30	75	30	0.055
6	70	75	30	0.065
7	30	75	60	0.063
8	70	75	60	0.09
9	50	70	30	0.062
10	50	80	30	0.057
11	50	70	60	0.085
12	50	80	60	0.085
13	50	75	45	0.075
14	50	75	45	0.08
15	50	75	45	0.082

A Ultrasonic Power

B Ultrasonic Pulser

C Ultrasonic Timer

APPENDIX E

FREE FATTY ACID ANALYSIS

Tabel E.1 Free Fatty Acid of Microalgae Oil

Run	Time (min)	% Ethanol (96%)	Algaeoil (before evp)	Algaeoil (after evp) 110^{o}C, 40 min	Sample (g)	Sample (g)	Vol Titration (ml)	% FFA
1	100	70	12.5	8	0.753	1.006	3.9	8.6
2	100	90	10.5	7.2	0.775	1.020	6.9	15.9
3	200	70	17.5	13	0.777	1.504	6.3	9.8
4	200	90	12	5	0.749	1.057	19	42.3
5	80	80	14	9	0.756	1.015	10	23.2
6	220	80	16	11.4	0.752	1.045	9.3	20.9
7	150	66	24	11	0.766	1.016	4.7	10.9
8	150	94	10	14,5	0.778	1.028	7	16.0
9	150	80	14	8.2	0.765	1.047	11.1	24.9
10	150	80	14.5	8.5	0.747	1.032	10.6	24.2

CALCULATION

Percent of Free Fatty Acid (%FFA)

Formula

$$\%FFA = \frac{\text{ml NaOH x N x MMfa}}{\text{Sample x 1000}} x\ 100$$

Run 1

$$\%FFA = \frac{3.9 \text{ x } 0.1 \text{ x } 235.4}{1.062 \text{ x } 1000} x\ 100$$

$$\%FFA = 8.6\%$$

Run 2

$$\%FFA = \frac{6.9 \text{ x } 0.1 \text{ x } 235.4}{1.020 \text{ x } 1000} x\ 100$$

$$\%FFA = 15.9\%$$

Run 3

$$\%FFA = \frac{6.3 \text{ x } 0.1 \text{ x } 235.4}{1.504 \text{ x } 1000} x\ 100$$

$$\%FFA = 9.8\%$$

Run 4

$$\%FFA = \frac{19 \text{ x } 0.1 \text{ x } 235.4}{1.057 \text{ x } 1000} x\ 100$$

$$\%FFA = 42.3\%$$

Run 5

$$\%FFA = \frac{10 \text{ x } 0.1 \text{ x } 235.4}{1.015 \text{ x } 1000} x\ 100$$

$$\%FFA = 23.2\%$$

Run 6

$$\%FFA = \frac{9.3 \text{ x } 0.1 \text{ x } 235.4}{1.045 \text{ x } 1000} x\ 100$$

$$\%FFA = 20.9\%$$

Run 7

$$\%FFA = \frac{4.7 \text{ x } 0.1 \text{ x } 235.4}{1.016 \text{ x } 1000} x\ 100$$

$$\%FFA = 10.9\%$$

Run 8

$$\%FFA = \frac{7.0 \text{ x } 0.1 \text{ x } 235.4}{1.028 \text{ x } 1000} x\ 100$$

$$\%FFA = 16.0\%$$

Run 9

$$\%FFA = \frac{11.1 \text{ x } 0.1 \text{ x } 235.4}{1.047 \text{ x } 1000} x\ 100$$

$$\%FFA = 24.9\%$$

Run 10

$$\%FFA = \frac{10.6 \text{ x } 0.1 \text{ x } 235.4}{1.032 \text{ x } 1000} x\ 100$$

$$\%FFA = 24.2\%$$

Average of %FFA

$$\%FFA = \frac{\text{total \%FFA}}{10}$$

$$\%FFA = \frac{196.7}{10}$$

$$\%FFA = 19.6\%$$

APPENDIX F

SAPONIFICATION NUMBER

Tabel F.1 Saponification Number of Microalgae Oil

Run	**Time (min)**	**% Ethanol (96%)**	**Algaeoil (before evp)**	**Algaeoil (after evp) 110^{o}C, 40 min**	**Sample (g)**	**Vol Titration (ml)**	**Vol Titration Blanko (ml)**	**Saponif Number**
1	100	70	12.5	8	0.753	21.5	29.2	286.8
2	100	90	10.5	7.2	0.775	27.7	29.2	54.3
3	200	70	17.5	13	0.777	24.3	29.2	176.9
4	200	90	12	5	0.749	26.3	29.2	108.6
5	80	80	14	9	0.756	26.9	29.2	85.3
6	220	80	16	11.4	0.752	27.7	29.2	55.9
7	150	66	24	11	0.766	24.8	29.2	161.1
8	150	94	10	14,5	0.778	20.5	29.2	108.1
9	150	80	14	8.2	0.765	27.1	29.2	77
10	150	80	14.5	8.5	0.747	27.9	29.2	28.5

CALCULATION

SAPONIFICATION NUMBER

Formula

$$\text{Saponification Number} = 28.05 \text{ x } \frac{(\text{titration blanko} - \text{titration sample})}{\text{wight of sample}}$$

Run 1

$$SN = 28.05 \text{ x } \frac{(29.2 - 21.5)}{0.753}$$

$$SN = 286.8 \frac{KOH}{1g \text{ oil}}$$

Run 2

$$SN = 28.05 \text{ x } \frac{(29.2 - 27.7)}{0.775}$$

$$SN = 54.29 \frac{KOH}{1g \text{ oil}}$$

Run 3

$$SN = 28.05 \text{ x } \frac{(29.2 - 24.3)}{0.777}$$

$$SN = 176.89 \frac{KOH}{1g \text{ oil}}$$

Run 4

$$SN = 28.05 \text{ x } \frac{(29.2 - 26.3)}{0.747}$$

$$SN = 108.60 \frac{KOH}{1g \text{ oil}}$$

Run 5

$$SN = 28.05 \text{ x } \frac{(29.2 - 26.9)}{0.756}$$

$$SN = 85.33 \frac{KOH}{1g \text{ oil}}$$

Run 6

$$SN = 28.05 \text{ x } \frac{(29.2 - 27.7)}{0.752}$$

$$SN = 55.95 \frac{KOH}{1g \text{ oil}}$$

Run 7

$$SN = 28.05 \text{ x } \frac{(29.2 - 24.8)}{0.766}$$

$$SN = 161.12 \frac{KOH}{1g \text{ oil}}$$

Run 8

$$SN = 28.05 \text{ x } \frac{(29.2 - 20.5)}{0.778}$$

$$SN = 108.16 \frac{KOH}{1g \text{ oil}}$$

Run 9

$$SN = 28.05 \text{ x } \frac{(29.2 - 27.1)}{0.765}$$

$$\text{SN} = 77 \frac{\text{KOH}}{\text{1g oil}}$$

Run 10

$$\text{SN} = 28.05 \text{ x } \frac{(29.2 - 27.9)}{0.747}$$

$$\text{SN} = 28.55 \frac{\text{KOH}}{\text{1g oil}}$$

Average of Saponification Number

$$SN \frac{\text{total SN}}{10}$$

$$SN = \frac{1142.33}{10}$$

$$\text{SN} = 114.233 \frac{\text{KOH}}{\text{1g oil}}$$