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

BUDI WIYARNO

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Faculty of Chemical and Natural Resources Engineering
UNIVERSITI MALAYSIA PAHANG

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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%.

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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
$\mathrm{P_a}$	Acoustic pressure
$\mathrm{P_h}$	Normal atmospheric pressure
$\mathrm{P_L}$	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.

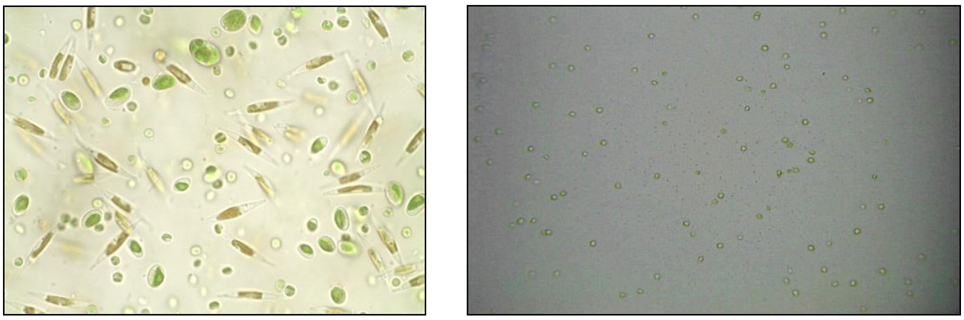
In attempt to solve the problem above, exploration of new energy sources needs to be done. One of the alternative energy sources that can be proposed is biofuel. Biofuel can be categorized as a renewable energy. Although the scale is relatively small in number, nowadays approximately 13% of world’s energy 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.

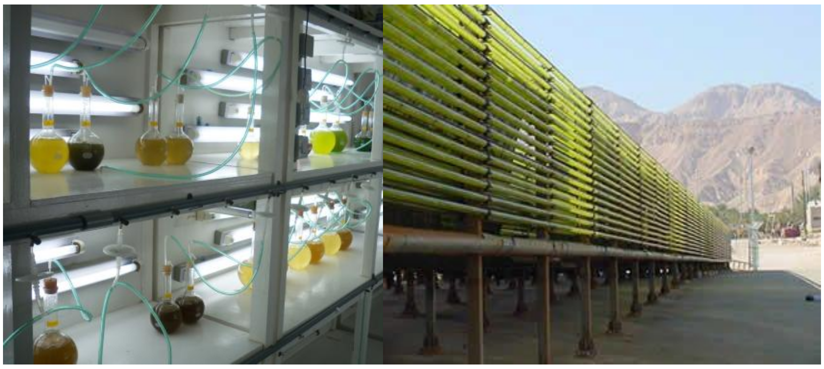
Each microalga has different specifications of media to grow, but in general microalgae can grow in a media which acidity level is pH 7-9. The temperature is approximately 20-30^{o}C. The temperature which is lower than 16^{o}C will cause the microalgae to grow slower. Meanwhile, the temperature which is more than 35^{o}C 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.