



# Biodiesel Production from Microalgae by Extraction – Transesterification Method

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**Abstract** - The environmental impact of using petroleum fuels has led to a quest to find a suitable alternative fuel source. In this study, microalgae were explored as a highly potential feedstock to produce biodiesel fuel. Firstly, algal oil is extracted from algal biomass by using organic solvents (n-hexane). Lipid is contained in microalgae up to 60% of their weight. Then, Biodiesel is created through a chemical reaction known as transesterification between algal oil and alcohol (methanol) with strong acid (such as  $H_2SO_4$ ) as the catalyst. The extraction – transesterification method resulted in a high biodiesel yield (10 % of algal biomass) and high FAMES content (5.2 % of algal biomass). Biodiesel production from microalgae was studied through experimental investigation of transesterification conditions such as reaction time, methanol to oil ration and catalyst dosage which are deemed to have main impact on reaction conversion efficiency. All the parameters which were characterized for purified biodiesel such as free glycerin, total glycerin, flash point, sulfur content were analyzed according to ASTM standard

**Key words** - Biodiesel, microalgae, extraction – transesterification, fatty acid methyl esters (FAMES), ASTM standard Doi: <http://dx.doi.org/10.12777/wastech.1.1.2013.6-9>

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## 1. Introduction

World faces the progressive depletion of its energetic resources mainly based on non-renewable fuels. Thus the unprecedented increase in energy consumption drives the rising petroleum fuel cost. In addition, the intensive utilization of fossil fuels has led to the increase in the generation of polluting gases released into the atmosphere, which have caused changes in the global climate. The solution to this issue depends on how the development and implementation of technologies based on alternative sources of energy will be undertaken. Through the use of renewable energetic resources, humankind can find part of the solution to their energy requirements in an environmentally friendly way.

One renewable solution is the use of solar energy in form of biomass (bioenergy). Conversion of these feedstock into biofuels is an important choice for the explosion of alternative energy sources and reduction of polluting gases. Microalgae are currently considered to be one of the most promising alternative and renewable feedstock sources for biofuels. In recent years, the “algae for fuel” concept has gained renewed interest with energy prices fluctuating

widely (Chisti, 2007; Hu et al, 2008). Not only the oil content in microalgae is slightly higher than that in other terrestrial plants, but also the algal biomass productivity is by far the highest.

In the algal biodiesel processes, fatty acid methyl esters (FAMES), the chemical composition of biodiesel, are commonly created by transesterification of algal oil with methanol using strong acid sulfuric ( $H_2SO_4$ , 98%) as a catalyst and n-hexane as a solvent. Different from terrestrial oil feedstock (such as palm, soybean or canola seed), algal oil is easily extracted from algal cell by crushing followed with solvent extraction. Mechanical crushing of algal biomass also can be used but it is really difficult to extract algal oil with the existing equipment.

There are several methods to prepare biodiesel from algal biomass (such as oil extraction from algal biomass by transesterification and direct transesterification from algal biomass (Johnson, 2009). Most extraction methods are based on method which was developed by Blich and Dryer in 1959 (Lewis et al, 2000). There are a number of steps producing biodiesel from microalgae. Firstly, the oils were extracted from algal biomass through a number of ways. The least expensive extraction is simply though cold

pressing (Johnson, 2009). Up to 70% of the oil contained within the algae can be extracted this way (Danielo, 2005). The use of organic solvent can increase this extraction level to 99%, but there is an increased cost in processing to achieve this (Metzger and Largeau, 2005).

In this study, extraction – transesterification method was used as a main way to produce biodiesel fuel from algal biomass. More specifically, this paper also focused on transesterification conditions such as reaction time, the methanol to oil ration, catalyst loading which have important role on transesterification reaction conversion efficiency.

## 2. Materials and experiment methods

### 2.1 Materials

Algae grow in most aquatic environment and need a light source, carbon dioxide, water and inorganic to create biomass. The growth medium must contain essential nutrients such as nitrogen, phosphorus, iron and sometimes silicon (Grobbelaar, 2004). In this study, algae are cultivated in pilot – open pond by domestic wastewater in Ho Chi Minh City. Algal have ability to treat many contaminants, but have proven to be great options when nutrients such as nitrogen (N) and phosphorus (P) need to be eliminated. The percentage of nitrogen and phosphorus reduction was 89.47% and 86.87% respectively. Biomass purity and productivity were affected by contamination with unwanted algal species and organisms that feed on algae. To harvest algae from this dilute solution, algal cells in solution are usually concentrated by sedimentation; the condensed slurry is then centrifuged at 4000 rpm for 15 minutes to further remove water. After centrifugation, the water content of the wet biomass was 80%. Depending on the experimental design in this study, the cell pellets were wet biomass.

Algal biomass contains three main components: carbohydrates, protein and lipids/natural oil. Because the bulk of the natural oil made by microalgae is in the form of triacylglycerides (TAGs) which is the right kind of oil for producing biodiesel, microalgae is the exclusive focus in the algae – to – biofuel arena (Danielo, 2005). The fatty acids attached to the TAG within the algal cells can be both short and long chain hydrocarbons. The shorter chain length acids are the idea for the creation of biodiesel, and some of the longer ones can have other beneficial uses.

For the extraction – transesterification method, Methanol, n-hexane, sulfuric acid (H<sub>2</sub>SO<sub>4</sub>), sodium chloride and other chemicals were purchased from Bach Khoa Co. Ltd (Ho Chi Minh, Viet Nam).

### 2.2 Method

The algal oil extraction procedure was adapted from the protocol described by Bligh and Dryer in 1959. Freeze – dried algal biomass (10 g) or wet biomass (with 10 g dry weight equivalent) was placed in the glass vial. Organic

solvent (n-hexane) and methanol were then added to the glass. The mixture was blended for 5 minutes, and then transferred to centrifuge tubes. After that, glass was washed twice by solvent and distilled water respectively. These mixtures also were added to the tube. The contents were then centrifuged at 4,000 rpm for 15 minutes. The organic layer containing algal oil was collected and transferred to a pre – weighed glass vial.

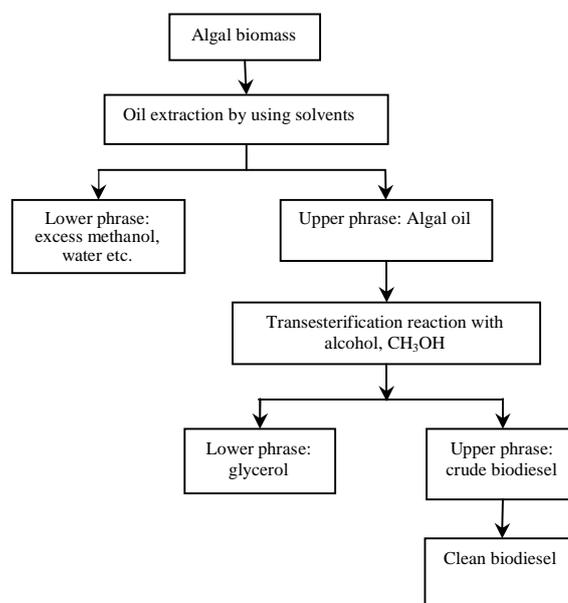


Fig.1 Schematic diagram showing the experimental steps typically undertaken for laboratory – scale production of microalgae biodiesel using an organic solvent mixture as a lipid extraction technology

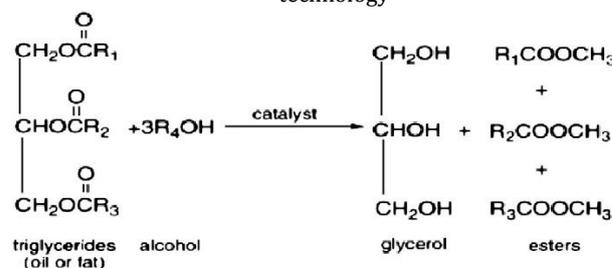


Fig. 2 Acid – catalyzed transesterification of triglycerides

The algal oil was heated at 75°C for 5 minutes to evaporate water content. A mixture of methanol, sulfuric acid and solvent (n-hexane) was added to the algal oil, and then the reaction mixture was blended for 35 minutes at a temperature of 75°C. After the reaction was completed, the samples were cooled down to room temperature, the crude ester layer (the upper phase) was separate from glycerol layer in a separating funnel. The raw ester layer contained methyl ester, possibly un-reactive oil, methanol and glycerol. To separate methanol, the organic layer was washed two times with distilled water in separating funnel

until the washings were neutral. The FAMES layer was dried by using NaCl solution. The upper phase containing FAMES was collected and transferred to a pre-weighed glass test tube. The solvent was removed by using Rotary Evaporator at 320 mbar and 36°C. After n-hexane eliminated, the composition of FAMES contained in the crude biodiesel fuel was analyzed by gas chromatograph (GC). According to theory, the transesterification requires 3 mol of alcohol per one mol of triglyceride to produce 3 mol of fatty acid methyl esters and 1 mol of glycerol. Excess alcohol was used to keep reaction volume consistent and result in higher ester yield. In this study, we investigated the role of substrate ratio in transesterification reaction in solvent – free medium. Firstly, the reaction was conducted in 20, 25, 30, 35 and 45 minutes at 75°C. After the effect of reaction time was determined, It is continued conducting the reaction at 3:1, 3.2:1, 3.4:1, 3.6:1 and 3.8:1 methanol to dry algal biomass (vol./wt.) ratios at 75°C and the most effective reaction time. Finally, the effect of catalyst loading on algal biomass conversion efficiency at 0.5:1, 0.55:1, 0.6:1, 0.65:1 and 0.7:1 catalyst (H<sub>2</sub>SO<sub>4</sub> 98%) to dry biomass (vol./wt.) ratios were also discussed.

### 2.3 Gas – chromatography analysis

The biodiesel sample was taken to analyze the purity by gas chromatography (agilent 6890), equipped with an HP Innovax capillary column (30 m×0.25 mm) and a flame ionization detector (FID). Nitrogen was used as the carrier gas at a constant flow rate of 20 mL/min. The column oven temperature was programmed from 50 to 130°C (at the rate of 20°C/min) and held at 130°C for 5 min, then raised to 260°C at 2.5°C/min and maintained at this temperature for 10 min.

## 3. Results and Discussion

### 3.1 Effect of reaction time

Effect of reaction time on procedure of biodiesel from microalgae is shown in Fig. 3. The methyl ester yield increased as the length of reaction time was increased. More specifically, the FAMES content in biodiesel increased dramatically from 20-min to 35-min reaction time, after that, the FAMES yield kept rising very slowly and then practically constant above 5.2% at 45 min. The reaction approved to be in equilibrium is the reason why the rate rose quite slowly. The results indicate that the biodiesel quality (heating value, based on FAMES content) at 35-minute reaction time is significantly higher than that in others.

### 3.2 Effect of methanol to dry algal biomass ratio

The ratio of methanol to oil is one of the most important variables that affects conversion efficiency as well as production cost of biodiesel. The results were indicated in Fig. 4. With further increase in (vol./wt.) ratios at 3.6:1, the conversion efficiency went down gradually and stable at

3.8:1 ratio. According to the results which were presented in Fig. 4, 3.4:1 methanol to dry algal biomass (vol./wt.) is the most efficient in experimental process. With the methanol to algal biomass (vol./wt.) increasing from 3:1 to 3.2:1, the FAMES yield increased considerably. The FAMES content achieved is clearly the highest at 3.4:1 methanol to dry algal biomass (vol./wt.) ratio.

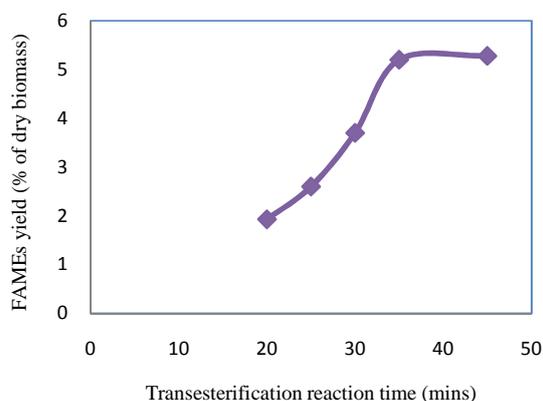


Fig. 3 – Effect of reaction time on algal biomass conversion efficiency. Under 3.4:1 methanol to dry biomass (vol./wt.) ratios, 0.6:1 catalyst to oil (vol./wt.) ratios at 75°C.

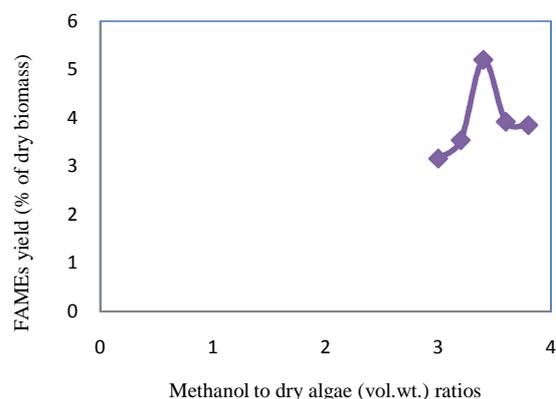


Fig. 4 - Effect of methanol to dry biomass (vol./wt.) ratios on algal biomass conversion efficiency. Under 0.6:1 catalyst to oil (vol./wt.) ratios at 75°C reacted in 35 minutes.

### 3.3 Effect of catalyst loading

The amount of catalyst which was used in the transesterification reaction is another important variable have effect on reaction rate. The FAMES yields of biodiesel at different catalyst to dry algae (vol./wt.) ratios were presented in Fig. 5. The conversion efficiency is by far the highest (5.2 %) at 0.6:1 catalyst to dry biomass (vol./wt.) ratio. From the ranges less than 0.6:1 (vol./wt), the catalyst loading was not enough to complete the reaction, so that the FAMES contents were significantly lower than that at 0.6:1 (vol./wt.) ratio. By contrast, the FAMES contents also decreased while the catalyst to dry biomass increased. Addition of excess amount catalyst, gave rise to the

formation of an emulsion, which increased the viscosity and led to the formation of gels (Xiangmei, 2008).

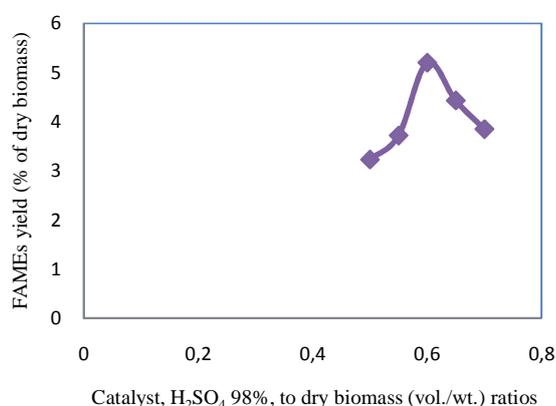


Fig. 5 - Effect of catalyst to dry biomass (vol./wt.) ratios on algal biomass conversion efficiency. Under 3.4:1 methanol to dry biomass (vol./wt.) ratios at 75°C reacted in 35 minutes.

### 3.4 FAMES analysis of biodiesel from microalgae

The FAMES in the hexane phase were analyzed using gas chromatograph equipped with a flame ionization detector. Calibration curves between peak area and concentration were established by injecting reference FAMES samples of known concentrations into the GC - FID. The fatty acid composition of the lipid from microalgae throughout the extraction - transesterification was shown in Fig. 6. The analysis of biodiesel sample indicated the occurrence of significant amounts of the methyl ester of octadecatrienoic acid (C18:3), hexadecanoic acid (C16:2) and octadecadienoic acid (C18:2). The total amount of these methyl esters made up 77% of the total FAMES yield.

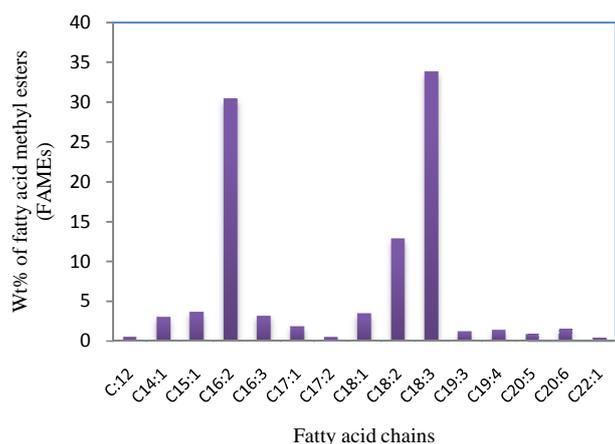


Fig. 6 FAMES analysis of biodiesel obtained from microalgae through the extraction - transesterification method at 35 min reaction time, 75°C.

## 4. Conclusion

It has been demonstrated that biodiesel can be produced from algal biomass through the extraction - transesterification method. The FAMES yield from microalgae was found to be significantly affected by the independent effects of three factors investigated. Throughout these experiments, biodiesel of good quality can be produced from microalgae in the following reaction conditions: 3.4:1 methanol to dry biomass (vol./wt.) ratio with 0.6:1 catalyst loading to dry algae (vol./wt.), temperature at 75°C in 35 minutes. Analysis of the methyl esters by gas chromatography indicated that the methyl ester of octadecatrienoic acid (C18:3) is by far the highest content.

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