

Preparation of Biodiesel from Microalgae and Palm Oil by Direct Transesterification in a Batch Microwave Reactor

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Abstract. The present work was aimed to study the so-called direct transesterification of microalgae lipids to biodiesel in a batch microwave reactor. As a comparison, preparation of palm oil to biodiesel by alkaline catalyzed ethanolysis was also carried out. Palm oil biodiesel was recovered close to an equilibrium conversion (94-96% yield) under microwave heating for at least 6 min, while the conventional method required more than 45 minutes reaching the same yield. A very short reaction time suggests the benefit of microwave effect over conventional heating method in making biodiesel. FTIR analysis revealed the presence of fatty acid ethyl esters with no undesired chemical groups or compounds formed due to local heat generated by microwave effect, thus the conversion only followed transesterification route. Oil containing microalgae of *Chlorella sp.* isolated from the local brackish water pond was used as a potential source of biodiesel. High yield of biodiesel (above 0.6 g/g of dried algae) was also attainable for the direct transesterification of microalgae in the microwave reactor. Effect of water content of the algae biomass became insignificant at 11.9 % (w/w) or less, related to the algae biomass dried for longer than 6 h. Fast transesterification of the algal oil towards equilibrium conversion was obtained at reaction time of 6 min, and at longer times the biodiesel yield remains unchanged. FAME profile indicates unsaturated fatty acids as major constituents. It was shown that microwave irradiation contributes not only to enhance the transesterification, but also to assist effective release of fatty acid containing molecules (e.g. triacylglycerol, free fatty acids and phospholipids) from algal cells. **Keywords:** biodiesel, transesterification, ethanolysis, microwave, palm oil, microalgae, *Chlorella sp.*

1. Introduction

Biodiesel is a non-petroleum-derived fuel that comprises alkyl esters produced by the transesterification of triacylglycerol. Biodiesel is renewable and environmental-friendly, with calorific value equivalent to petroleum-based fuel. Transesterification has been recognized as the simplest method to prepare biodiesel from edible and non-edible lipids. However, energy consumption inefficiencies in the process which result in higher cost of biodiesel is still a great concern. This is generally related to the heating method employed in the process. Recently, many investigations have applied microwave irradiation in biodiesel production due to inherent advantages of shorter reaction time and lower energy requirements found to complete numerous inorganic and organic chemical reactions [1]. Microwave assisted reactions not only reduce the reaction time and increase



the biodiesel yield but also could reduce use of alcohol and the product separation time significantly [2,3].

The present study deals with the use of microwave reactor operated non-isothermally to convert microalgae lipid to biodiesel by in-situ or direct transesterification, an approach that has been widely used as an analytical procedure to prepare FAME for the determination of the fatty acid composition of lipid containing tissues [4,5]. The direct transesterification method can produce FAME in higher quantity than can be achieved in the two-step method of traditional solvent extraction and transesterification [5,6,7]. The method was also successfully applied for pure and mixed cultures of microorganism. Therefore, a combination of microwave heating and direct transesterification is expected to enhance biodiesel yield from microalgae at a very short time. As a comparison, palm oil biodiesel was also prepared by alkaline catalyzed transesterification under conventional and microwave heating.

2. Experimental Method

2.1 Materials

Palm frying oil (Bimoli) was purchased from a local grocery store. It is the only branded frying oil sold bulky in the market. The green microalgae strain used in this experiment was *Chlorella sp.* which was collected from 5 L cultivation tanks. The alga was grown in domestic wastewater medium. Reagent grade methanol, chloroform, sulfuric acid, technical grade alcohol (96% ethanol), and sodium hydroxide were used as purchased without any further purification. Distilled water was used to wash biodiesel. The reaction was carried out in a 250 ml screw-capped bottle, placed inside a household microwave oven (Model NN-ST 342M, Panasonic) operating at frequency 2.45 GHz with maximum output 800 W.

2.2 Transesterification of Palm Oil

A ratio of oil and ethanol of 100:50 (ml/ml) and catalyst loadings of 0.25-1.0 g NaOH/100 ml of oil was used throughout the experiments. The oil, ethanol, and NaOH were charged into a 250 ml bottle with screw-cap. Vigorous mixing was applied for 2 minutes to obtain a good dispersion of oil-ethanol. The reaction bottle was placed inside the microwave oven with heating set at low-micropower. The reaction time was adjusted for 1 to 10 minutes without temperature control, thus the temperature rise was observed (referred to the temperatures measured at the end of each reaction time). Upon cooling to room temperature the reaction bottle was removed from the microwave and the reaction mixture was settled in a separatory funnel. The final biodiesel was obtained after the upper layer separated and washed with water three times. As a comparison, palm biodiesel was also prepared by conventional method; the transesterification in a stirred glass reactor by electric heating at 60°C for 15, 30 and 60 min. The stirring speed was maintained at 200 rpm. Biodiesel produced by microwave heating was characterized to determine density and viscosity. The chemical changes of the PO biodiesel were analyzed by Transform Infrared (FTIR) Spectrophotometer (Model 8400S, Shimadzu) equipped with Interferometer to eliminate the effect of H₂O and CO₂ in the surrounding atmosphere.

2.3 Direct Transesterification of Microalgae

Wet microalgae biomasses were oven-dried at varying times to obtain certain water content. Initial work on algae suggests that drying time of 6 h resulted in water content of 0.113 g/g biomass which did not significantly affect the biodiesel yield. Therefore, the rest experiments on microalgae used 6 h dried biomass. The microalgae was poured into a 250 ml reaction bottle containing a mixture of methanol, H₂SO₄, and chloroform (1.7:0.3:2.0 v/v/v) as prescribed by Indarti *et al.* [5]. The bottle was tightly closed with screw-cap and placed inside the microwave chamber. The mixture was heated by microwave irradiation set at low-micropower for a given time. After the heating treatment, the mixture was allowed to cool for 30 min and transferred to a separatory funnel. About 10 ml distilled water was added to the mixture, which was then shaken and allowed to separate. The yield of biodiesel was evaluated by gravimetric method. The bottom layer containing FAME was poured into a pre-weighed

petri dish, which was evaporated in an oven at 60°C overnight. The remaining liquid was weighed as biodiesel mass.

Fatty acid methyl ester (FAME) content of the algal biodiesel sample was determined with a gas chromatography-mass spectrometer (GC-MS) (Model QP2010 Plus, Shimadzu) equipped with flame ionization detector (FID) and an Rtx-5MS column (0.25 mm ID and 30 m long, Restek). One microliter of the bottom layer was injected into the GC unit. The fatty acids were identified by comparing the retention times with those of standard fatty acids. Their composition was calculated based on the corresponding peak areas in the chromatogram.

3. Results and Discussion

3.1 Preparation of Palm Oil Biodiesel under Microwave Heating

The ability of a molecule to couple with the microwave radiation is a function of its molecular polarisability (i.e. a function of its dipole moment), thus only polar molecules interact with microwave energy [1]. Palm oil and ethanol as reacting fluids involved in the transesterification are polar compounds and their interaction with the microwave radiation has resulted in temperature increase to more than 80°C after 10 min. The reaction time was then limited to 10 min to avoid excessive boiling of compounds in the reaction mixture.

Figure 1 indicates that the yield of biodiesel reached a maximum value in less than 10 min depending on the catalyst amount added. The maximum yield of 94-96% suggests the reaction was in the equilibrium. Beyond the maxima, a slight decrease in biodiesel yield observed probably due to the favor of the equilibrium in the reverse reaction. It is in good agreement with the findings of Hernando *et al.* [8] and Suppalakpanya *et al.* [9] in producing biodiesel from rapeseed oil and crude palm oil, respectively. Compared with the conventional heating method, the yields of biodiesel reached close to maximum after 45 min as shown in insert of Figure 1. A very short reaction time with microwave heating to obtain the optimum yield suggests the benefit of microwave over conventional heating method in making biodiesel.

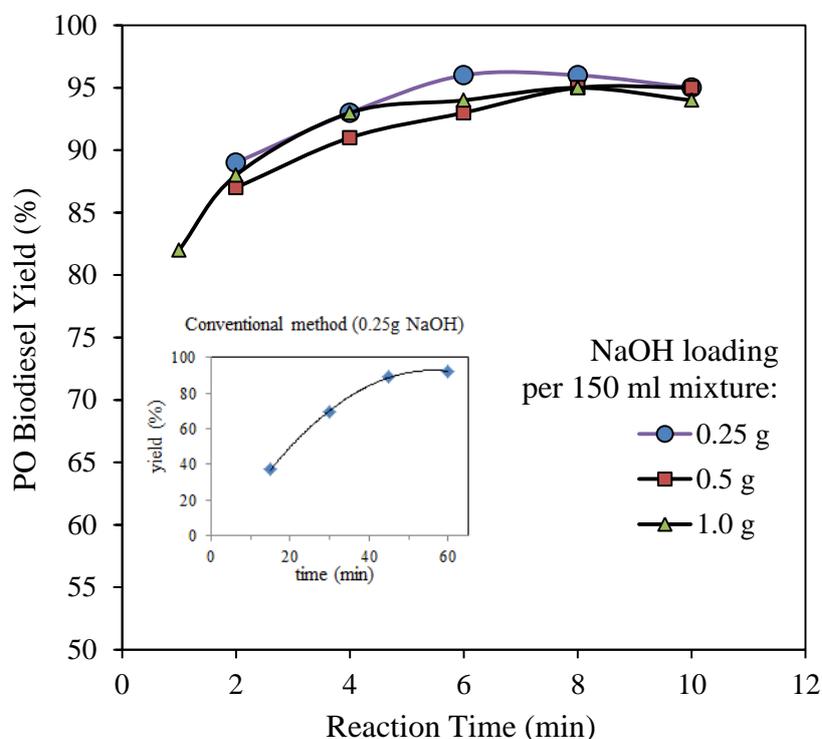


Figure 1. Change of PO biodiesel yield during microwave heating.

After the transesterification, the reaction mixture was allowed to separate into the upper layer containing biodiesel and the bottom layer. The upper layer was then washed with warm water three times. Further separation of dispersed phase was carried out by centrifugation. Later, the remaining biodiesel layer was washed again three times. The quality of palm biodiesel produced in this work was observed for its physical and chemical parameters.

The density of PO biodiesel at 40°C was 0.880-0.888 g/ml which is equivalent to petroleum-based diesel (0.834 g/ml) and within ASTM D6751 and SNI 04-7182-2006 limits for biodiesel. Viscosity has been the most crucial property of biodiesel since its effect on the fluidity of the fuel determines the fuel injection system, particularly at low temperature. The viscosity of PO biodiesel 40°C was 3.825-4.655 cps which is slightly higher than ASTM D6751 limit for biodiesel, but within the SNI 04-7182-2006 value for Indonesian biodiesel.

FTIR analysis has been shown as a versatile method to determine the quality of biodiesel [10,11]. Figure 2 shows that the FTIR spectra of origin oil and the produced biodiesel are very similar since the reaction did not cause significant change of chemical or functional bonds. The hot spot effect of microwave heating did not lead to undesired reaction routes. The triacylglycerol was broken down into fatty acid ethyl esters and glycerol. The FTIR spectra of the origin oil and biodiesel showed peaks from carbonyl (C=O) at 1744 cm⁻¹ and that of C-O at 1163 cm⁻¹ similar to that reported in the literature [12], indicating that the carbonyl and C-O in the oil is retained in the biodiesel. The stretching vibrations of CH, CH₂, and CH₃ appear at 3003, 2854, and 2922 cm⁻¹, whereas the bending vibrations (ρ CH₂) of these groups are found at 1375, 1163 and 723 cm⁻¹, respectively.

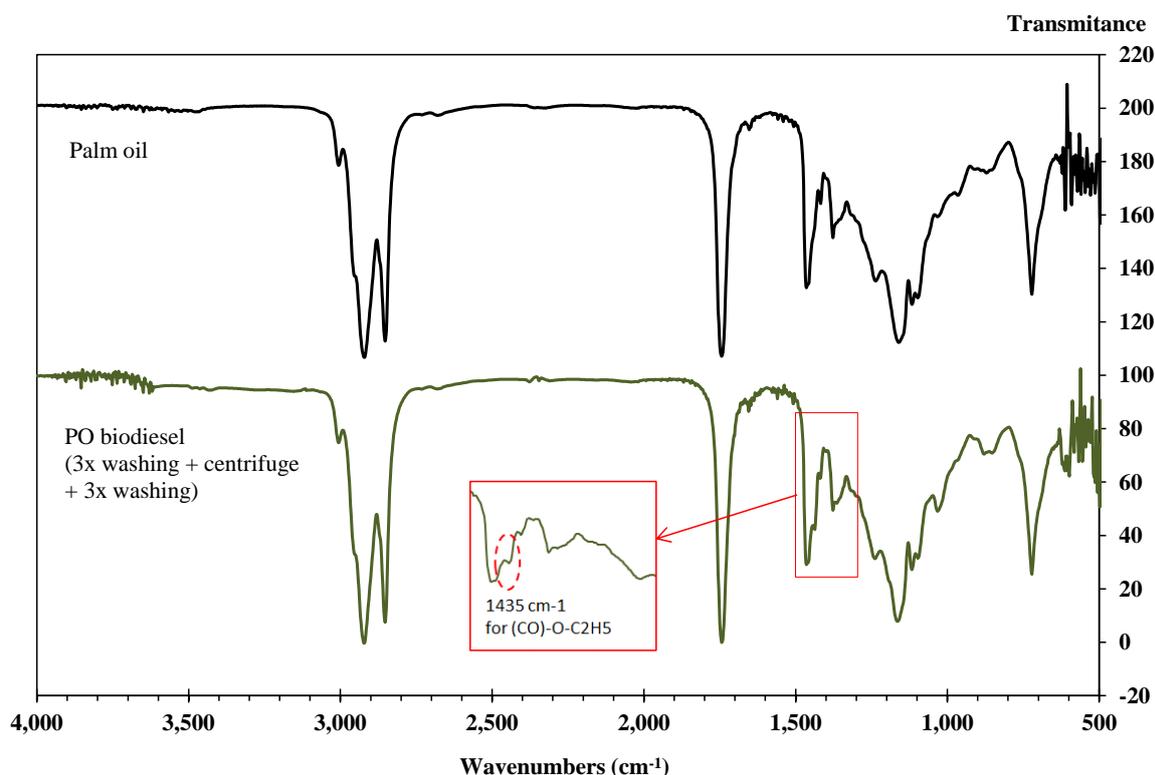


Figure 2. FTIR spectra of palm oil and PO biodiesel

Some specific peaks of biodiesel are possibly to be identified with close spectra observation as given in Figure 2. The most influence as a result from transesterification was to find the new signal at 1435 cm⁻¹ which is definitely the ethyl ester group with its deformation vibration (Shimadzu, SCA_110_035). Tahvildari and Mohammadi [13] in preparing ethyl esters from linseed oil reported

the shifting of absorption peaks of the oil sample at 1744 and 1162 cm^{-1} to 1739 and 1178 cm^{-1} in the biodiesel, respectively, but it was not clearly observed in this work.

3.2 Direct Transesterification of Microalgal Oil

Chlorella sp. used as a source of oil was isolated from brackish water pond in Tanjong Selamat (Aceh Besar) by serial dilution in the modified Detmer medium. The stock culture of *Chlorella sp.* was then maintained in BG-11 medium with light irradiation and CO_2 -enriched air flow. Previous study under optimized condition, the microalgae could accumulate crude lipid as high as 0.821 g/g dried biomass. For the purpose of this experiment, the algal biomass were then cultured in 5 L glass tanks filled with 50% diluted municipal domestic wastewater, and harvested in their stationary growth phase. The collected algal biomass was drained for 2 h before subject to drying.

Water present in the biomass has a damaging effect on the yield of biodiesel [14,15]. In the present work, different water content of the algal biomass was used to examine the water effect on the biodiesel yield from *Chlorella sp.* using the direct transesterification method. Freshly harvested biomass (after 2 h draining) contained water of 48.0% (wet basis). Figure 3 shows the change of water level of the algal biomass upon drying at 60°C for various times. The biomass was found completely dried after 12 h, assuming that the biomass was free of unbounded water. Water significantly inhibits the algal oil to biodiesel conversion with water content greater than 11.9%. When the water content less than 11.9%, biodiesel yield was relatively constant (0.627-0.629 g/g dried biomass). It is probably due to excess methanol of the reacting mixture minimizing water effect. Similar finding was also found in the direct transesterification of *Chaetoceros gracilis* since a higher water content of the algal biomass was compensated by adding more methanol [14].

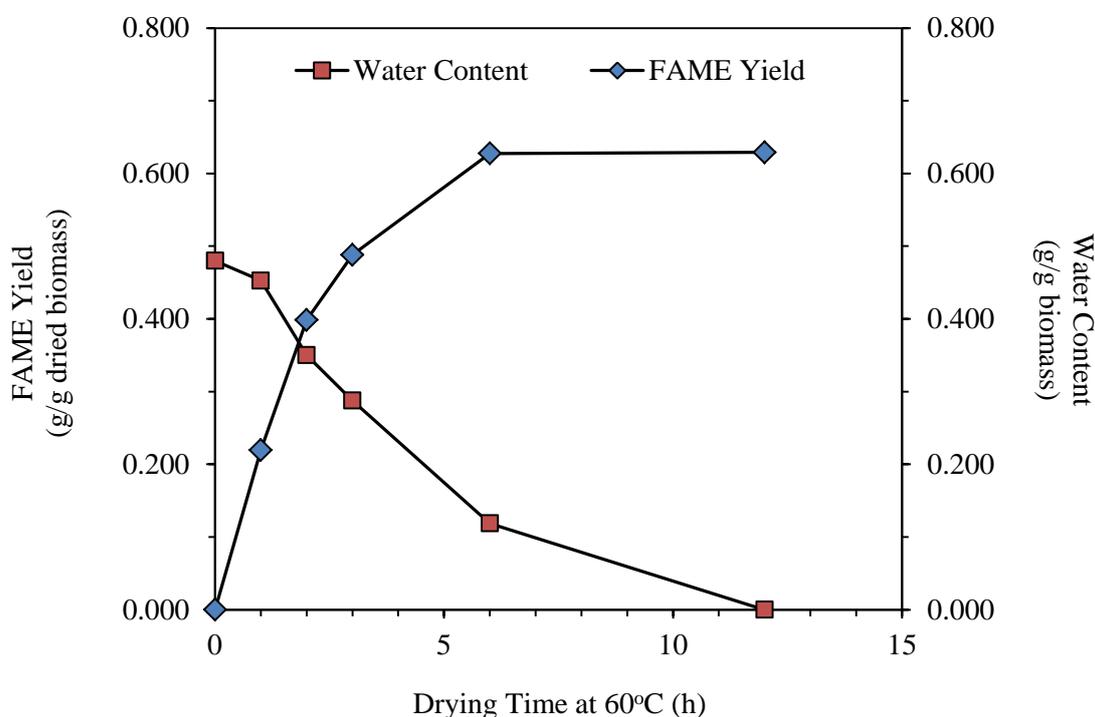


Figure 3. Effect of drying time on water content and the biodiesel yield (10 min reaction)

To investigate the influence of heating time, the algal biomass with a water content of 0.113 g/g of wet algae was used since it was found that no considerable differences in the biodiesel yield were observed with the use of lower water content. Figure 4 shows an increasing reaction time allows the transesterification reaction to proceed to completion and results in a higher yield of biodiesel from the

microalgae. A very short conversion time was also reached to produce the algal biodiesel as high yield was attained at least after the microwave heating of 6 min, which corresponding to the yield of 0.591 g of biodiesel per g of dried biomass. Higher amount of biodiesel yield (>0.620 g/g of dried biomass) was obtained at slightly longer reaction times. Patil *et al.* [16] found the optimum reaction time in preparing biodiesel from *Nannochloropsis* by microwave assisted direct transesterification was 4-5 min, since microwave irradiation contributes to more effective in the destruction of the cells and accelerating better the transesterification reaction in a shorter reaction time. With sulfuric acid and extraction agents (methanol and chloroform), 2 min was sufficient for cell disruption under microwave irradiation at 60°C [17]. However, longer reaction time to reach maximum yield was reported in transesterifying *Chlorella pyrenoidosa* (30 min) and *Chaetoceros gracilis* (more than 125 min) [14,18].

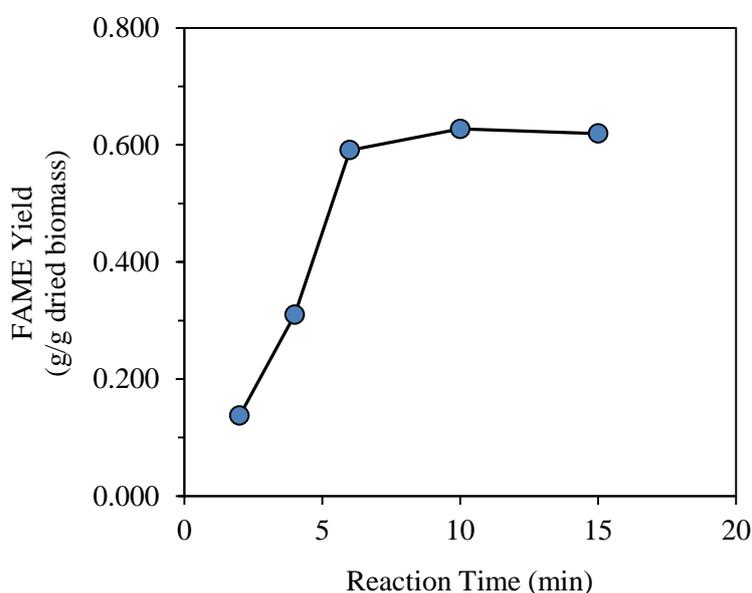


Figure 4. Effect of reaction time on the algal biodiesel yield

Fatty acids of the algal biodiesel were determined by GC analysis and given in Table 1. In general, FAME composing the algal biodiesel was not only from the triacylglycerol and the free fatty acids, but also from the other forms of lipid due to the extractable effect of methanol and chloroform as co-solvent in the direct transesterification. Both simultaneously dissolve phospholipids, glycolipids, sphingolipids, and sterols from cell membranes. Table 1 shows that the algal biodiesel comprises 28% saturated fatty acids and 72% unsaturated ones. The relatively high content of unsaturated fatty acids was also reported for other algal biodiesel [15,16,18]. Although fatty acid profile and degree of saturation does not have much of an impact on the transesterification reaction, they do affect the biodiesel properties and then determine its uses. Due to inherent properties of unsaturated fatty acids, biodiesel dominated by such fatty acids has good cold-flow properties. Unfortunately, these fatty acids are mostly vulnerable to oxidation. Biodiesel produced from *Chlorella sp.* cultivated for this work, therefore, may have an instability problem during storage for a long period.

Table 1. Fatty acid profile of the biodiesel derived from *Chlorella sp.*

No.	Fatty acids	Area (%)
1.	Myristic acid C14:0	3.7
2.	Palmitic acid C16:0	19.4
3.	Palmitoleic acid C16:1	2.4
4.	Oleic acid C18:1n9	18.0
5.	Linoleic acid C18:2n6	26.8
6.	Linolenic acid C18:3n3	24.7
7.	Arachidic acid C:20	4.9

4. Conclusion

A combination of microwave irradiation and direct transesterification was successfully implemented in converting palm oil and microalgal oil to biodiesel in high yield. Microalgae used were *Chlorella sp.* cultured in diluted municipal wastewater. Using pure oil such as palm oil, it was revealed that no undesired species was formed during microwave irradiation, thus the transesterification was the only reaction mechanism taking place. Transesterification of palm oil and microalgae took place at fast rate, reaching an equilibrium conversion within 6-10 min. When using microalgae biomass, water content should be taken into account. Water content of 11.9% (wet basis) or less was required to attain high biodiesel yield. High yield for the biodiesel from microalgae was achieved due to simultaneous contribution of fast transesterification, enhanced releases of lipids and role of chloroform as co-solvent during microwave irradiation. This study suggests that use of microwave in the direct transesterification of lipids contained in microalgae is an effective approach to reduce reaction time and costs as some operation steps are eliminated. Further cost reduction is also feasible by reducing feedstock cost as the microalgae can be cultured in wastewater streams or lagoon systems.

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