Research Article

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Advancing biodiesel production from microalgae *Spirulina* sp. by a simultaneous extraction–transesterification process using palm oil as a co-solvent of methanol

<https://doi.org/10.1515/chem-2020-0133>

received October 30, 2018; accepted May 17, 2020

Abstract: Microalgae have been considered as a potential candidate for biodiesel feedstock. Single-stage simultaneous extraction–transesterification process is proposed for simpler and more effective biodiesel conversion. In this study, the experiment of biodiesel production from microalgae *Spirulina* sp. was performed in a batch-stirred reactor using palm oil as a co-solvent of methanol and catalyzed by potassium hydroxide at a percentage of 1 wt% (w/w of palm oil). The effects of methanol–palm oil molar ratio, palm oil–microalgae weight ratio, and temperature on biodiesel yield were investigated. The results showed that the best biodiesel yield was 85.28% (99.01% of partial biodiesel yield from palm oil and 16.69% of partial biodiesel yield from dry microalgae), obtained at a methanol–palm oil molar ratio of 10:1, a palm oil–microalgae weight ratio of 5:1, and at a temperature of 60°C. Upon comparison, the overall yield increased by 34.59% (37.73% of partial biodiesel yield from palm oil and 13.00% of partial biodiesel yield from dry microalgae) than that of the two-stage (conventional) method. Single-stage simultaneous extraction–transesterification process also reduced the number of unsaturated fatty acid components in biodiesel that will lower the biodiesel quality.

Keywords: microalgae, *Spirulina* sp., biodiesel, simultaneous extraction–transesterification, palm oil–methanol mixture

1 Introduction

In the last few decades, the rapid growth of population and technology has led to the increase in energy demand in the world [1]. To meet this requirement, fossil fuels are still in the top rank of reliable energy resources [2]. However, the limited availability of fossil fuels has driven all stakeholders to develop alternative and sustainable fuels [3]. They are vigorously promoted for replacing conventional fuels and overcoming energy crisis with less environmental impacts [4]. The latest environmental issue related to the encouragement of these fuels is greenhouse gas effect reduction, holding world's temperature increase [5] and making a better living environment [6].

Recently, biodiesel has attracted public attention as a propitious biofuel for replacing fossil-based diesel fuel because of its renewable, sustainable, and environmentally friendly properties [7]. The main content of biodiesel is ester compounds from fatty acids, which are generally consisted in vegetable oils and converted into esters by transesterification with short-chain alcohols (methanol or ethanol) in the presence of catalyst [8]. At the first generation, fatty acids commonly used for biodiesel production are from edible oils, such as palm oil [9], soybean oil [10], corn oil [11], and sunflower oil [12]. However, some problems are generated because it can inflict a conflict with food sector, causing a higher global food price and threatening global food security [13]. Moreover, the requirement of large land for oil crop plantation is another threat on utilizing this resource [14].

As a solution to these problems, many researchers have explored non-edible oil and waste for biodiesel production, called the second generation of biodiesel feedstock. Kusumaningtyas et al. [15] studied esterification of *Jatropha* oil for fatty acid ethyl ester production. Ullah et al. [16]

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synthesized biodiesel from waste cooking oil using acid-based ionic catalysts. Wijaya *et al.* [17] transesterified the used cooking oil accelerated using heterogeneous catalyst $K_2O/MCM-41$. Sawitri *et al.* [18] also reported esterification of palm fatty acid distillate, waste from palm oil refinery, for producing fatty acid methyl esters (FAMES). Although more pre-treatments were conducted to the second feedstock, biodiesel still required more separation processes to remove impurities and ensure its quality with the standard. Therefore, microalgae have been recently considered as a potential candidate to produce biodiesel.

Microalgae are single-cell organisms, which are active in photosynthesis producing carbohydrates, lipids, and proteins [19]. They have considerable advantages compared to oil crops as follows: (i) higher growth rate [20], (ii) less cultivation area requirement [21], (iii) higher lipid content [22], (iv) higher ability of carbon capture and storage [23], and (v) more adaptive to low-quality medium (even waste water) [23]. In addition, biodiesel resulting from microalgae has similar properties to diesel fuel [24]. Despite the aforementioned advantages, the sequential stages of algal oil extraction and transesterification still become an obstacle on conventional microalgae-based biodiesel production, resulting in inter-process complexity [25] and high production cost [26]. To minimize the effects of it, a single-stage simultaneous extraction–transesterification process is offered.

Single-stage simultaneous extraction–transesterification (*in situ* or direct transesterification) is a process of biodiesel production directly from biomass by integrating oil extraction and transesterification process in a single reactor [27]. This process is expected to improve the process effectivity and reduce the use of chemicals [28]. The chemicals commonly used are methanol [29], hexane [30], and chloroform combined with hexane [31]. In spite of producing biodiesel in high yield, the effect of their toxicity is one of the concerned reasons to search other harmless chemicals. Based on the issue, palm oil is proposed to examine its performance in this process. Palm oil is predicted to extract algal oil effectively due to its similar components and its polarity. Likewise, palm oil can reduce the saponification process as microalgae consist of high free fatty acid. Therefore, mixing palm oil–methanol and microalgae is identified to increase biodiesel yield.

This work investigated simultaneous extraction–transesterification of *Spirulina* sp. into biodiesel by using palm oil as a co-solvent of methanol. Palm oil was chosen as a co-solvent because its phase (organic) was hypothetically similar to algal oil. Based on the similar phase, algal oil would be easier to be extracted by palm oil before converted into biodiesel using homogeneous

KOH catalyst. The performance of palm oil–methanol mixture was evaluated by measuring the overall biodiesel yield and calculating the partial biodiesel yield from palm oil and dry microalgae. Parameters evaluated in this study were methanol–palm oil molar ratio, palm oil–microalgae weight ratio, and temperature. The results were then compared with a two-stage (conventional) method, where algal oil was extracted by palm oil and then transesterified with methanol for biodiesel production. This article also discussed the comparison of this result with previous findings.

2 Materials

The dried powder biomass of microalgae *Spirulina* sp. was supplied by CV. Blue Green Algae, Lamongan, Indonesia. The chemical composition, proximate, elemental, and heating value characteristics of this alga are listed in Table 1. Algal oil was extracted from the algal cell using *n*-hexane as a solvent, and then its fatty acid composition was characterized by gas chromatography-mass spectrometry (GC-MS). The solvent used in this study was a mixture of palm oil and methanol. Palm oil of refined grade was supplied by PT. Salim Ivomas Pratama, Tbk. The physical properties of palm oil are represented in Table 2. Hence, to determine its fatty acid

Table 1: Characteristics of microalgae *Spirulina* sp. [32]

Component	Composition
Chemical composition (%)	
Carbohydrate	46.13
Lipid	0.25
Protein	44.72
Proximate (%)	
Fixed carbon	12.51
Volatiles	67.03
Moisture	11.83
Ash	8.63
Elemental (%)	
Carbon (C)	41.91
Hydrogen (H)	6.82
Oxygen (O)	33.04
Nitrogen (N)	8.89
Sulfur (S)	0.49
H/C molar ratio	1.95
O/C molar ratio	0.59
Calcium (Ca)	0.91
Magnesium (Mg)	2.70
Potassium (K)	0.17
Higher heating value (MJ/kg)	20.09

Table 2: Physical properties of palm oil [33]

Properties	Value	Method
Density at 40°C (kg/m ³)	898.50	ASTM D-4052-11
Kinematic viscosity at 40°C (cSt)	44.97	ASTM D-445
Water content (wt%)	0.1940	ASTM D-4377
Free fatty acid content (wt%)	0.0550	Titrimetric
Acid value (mg KOH/g oil)	0.1095	ASTM D-664
Iodine value (wt%)	59.39	AOCS Cd 1-25

composition, palm oil was analyzed using GC-MS. Methanol at a concentration of more than 99.8% was purchased from CV. Genera Labora, Yogyakarta, Indonesia. In addition, potassium hydroxide, produced by Merck, was also required for accelerating transesterification.

3 Methods

3.1 Two-stage method procedures

The experiment of two-stage method (extraction and transesterification) was conducted using a batch stirred reactor. This reactor consisted of a 500 mL three-necked round-bottom flask, a heater, a mechanical stirrer, and a thermometer under the reflux system, as shown in Figure 1. First, 300 g of palm oil was heated and stirred at 750 rpm inside the reactor, attaining 50°C of temperature. After reaching the desired temperature, 60 g of dried microalgae was put into the palm oil flask. This process was performed under temperature control for 2 h. Then, the mixture was filtered under vacuum conditions, obtaining separated liquid and solid phases.

The liquid phase was then inserted again into the reactor and heated to reach a temperature of 50°C. In the

Figure 1: Experimental equipment set [33].

other flask, 120 mL of methanol was mixed with 1 wt% (w/w of palm oil) of potassium hydroxide to produce methoxy solution at the same temperature with palm oil. The methoxy solution was then added into the reactor and stirred under temperature control for 2 h. Then, the mixture was first decanted to separate organic and inorganic liquids. The organic liquid was then washed using warm distilled water (40–50°C) followed by second decantation to remove the water. Next, the organic liquid was heated to remove the remaining water and is ready for analysis.

3.2 Simultaneous extraction–transesterification procedures

The experiment procedures of simultaneous extraction–transesterification were similar, but simpler than those of the two-stage method. A total of 300 g of palm oil, certain methoxy solution (methanol–palm oil molar ratios of 8:1, 10:1, and 12:1), and certain microalgae powder (palm oil–microalgae weight ratios of 3:1, 4:1, 5:1, and 6:1) were reacted in one stage for 2 h at desired temperatures (40, 50, and 60°C) and a stirring rate of 750 rpm. Then, the mixture was filtered under vacuum conditions, obtaining separated liquid and solid phases. Warm distilled water (40–50°C) was then added to the liquid phase and it was followed by decantation to remove inorganic liquid. Furthermore, the biodiesel layer was then heated to remove the remaining water and is ready for analysis.

3.3 Product analysis

The performance evaluation of this advanced process was conducted by measuring biodiesel yield and analyzing FAMES in biodiesel. For biodiesel yield, the total mass of biodiesel was measured after removing the water content in biodiesel. Furthermore, FAME composition analysis of biodiesel was carried out using GC-MS. The type of GC-MS used was SHIMADZU QP2010S, which used AGILENT HP-5 as a column type (inner diameter = 0.25 mm; length = 30 m) and helium as a carrier gas. Prior to GC-MS analysis, biodiesel samples were dissolved in hexane. For palm and algal oils, the fatty acid components in samples must be converted into FAMES by mixing them with methanol and BF₃ at a temperature of 60°C for 1 h before extracting FAMES using hexane.

3.4 Data analysis

The overall and partial biodiesel yields were evaluated. The overall biodiesel yield was determined by comparing mass of total biodiesel (m_{bio}) with sum of palm oil (m_{palm}) and dry microalgae initial mass (m_{dm}), as shown in the following equation:

$$\text{Overall yield (\%)} = \frac{m_{\text{bio}}}{(m_{\text{palm}} + m_{\text{dm}})} \times 100\%. \quad (1)$$

Meanwhile, partial biodiesel yields from palm oil ($\text{Yield}_{\text{palm}}$) and dry microalgae (Yield_{dm}) were optimized by fitting overall yield data and FAME composition data using MATLAB (The Math Work, Inc.). The formulas for supporting this calculation are expressed in equations (2)–(5).

$$X_{i,\text{palm}} \times m_{\text{palm-bio}} + X_{i,\text{algal}} \times m_{\text{algal-bio}} = X_{i,\text{bio}} \times m_{\text{bio}}, \quad (2)$$

$$m_{\text{palm-bio}} + m_{\text{algal-bio}} = m_{\text{bio}}, \quad (3)$$

$$\text{Yield}_{\text{palm}} (\%) = (m_{\text{palm-bio}}) / (m_{\text{palm}}) \times 100\%, \quad (4)$$

$$\text{Yield}_{\text{dm}} (\%) = (m_{\text{algal-bio}}) / (m_{\text{dm}}) \times 100\%, \quad (5)$$

where $X_{i,\text{palm}}$ denotes the mass fraction of FAME “ i ” in palm oil, $m_{\text{palm-bio}}$ denotes the mass of biodiesel-converted palm oil, $X_{i,\text{algal}}$ denotes the mass fraction of FAME “ i ” in algal oil, $m_{\text{algal-bio}}$ denotes the mass of biodiesel-converted dry microalgae, and $X_{i,\text{bio}}$ denotes the mass fraction of FAME “ i ” in total biodiesel. The iteration of $\text{Yield}_{\text{palm}}$ and Yield_{dm} was run by using the “fsolve” program in MATLAB.

Ethical approval: This research is not related to either human or animal use.

4 Results

4.1 Fatty acid composition of palm and algal oils

Palm oil is a triglyceride containing several types of saturated fats, such as lauric acid (0.1%), myristic acid (1%), stearic acid (5%), and palmitic acid (44%). Palm oil also consists of unsaturated fats in the form of oleic acid (39%), linoleic acid (10%), and alpha linoleic acid (0.3%) [34]. Because of its content, palm oil is generally utilized as a raw material for making biodiesel in a large scale. However, this process has aroused several issues as previously elucidated. To overcome these obstacles, microalgae as the third biodiesel generation

Figure 2: Major fatty acid component in palm and algal oils.

have been broadly employed for producing biodiesel owing to 40–80% lipid content [34]. The composition of fatty acid both in palm oil and microalgae is presented in Figure 2.

4.2 Comparison of two-stage method and simultaneous extraction–transesterification

The present study compared two-stage method and simultaneous extraction transesterification by comparing FAME composition differences and calculating the yield of each method. The composition of FAME component in biodiesel generated from each method is first depicted in Figure 3. Then, the performance difference of both methods was evaluated by comparing overall biodiesel yield, partial biodiesel yield from palm oil, and partial biodiesel yield from dry microalgae, as shown in Table 3.

4.3 Effect of methanol–palm oil molar ratio

The observed effect of methanol–palm oil molar ratio on FAME composition and biodiesel yield of simultaneous extraction transesterification is shown in Figure 4 and Table 4.

4.4 Effect of palm oil–microalgae weight ratio

The observed effect of palm oil–microalgae biomass weight ratio on FAME composition and biodiesel yield of

Figure 3: Major FAME composition of biodiesel generated by two-stage method and simultaneous extraction transesterification.

simultaneous extraction transesterification is shown in Figure 5 and Table 5.

4.5 Effect of temperature

The observed effect of temperature on FAME composition and biodiesel yield of simultaneous extraction transesterification is shown in Figure 6 and Table 6.

5 Discussion

5.1 Fatty acid composition of palm and algal oils

Based on Figure 2, it reveals that fatty acid compounds in microalgae have the same characteristics as palm oil except for myristic acid component. Due to the similarity of polar properties, the utilization of palm oil can potentially increase the biodiesel yield from microalgae biomass. Likewise, palm oil has the potential to be further developed as a novel research for producing biodiesel as it has two main roles in the process, acting as a raw material and a co-solvent.

Although similar in type of major components, the level of methyl oleate and the methyl palmitate content in palm and algal oils were different. The methyl myristate content of palm and algal oils was 1.46% and trace, respectively. The methyl palmitate content of palm and algal oils was 37.37% and 44.76%, respectively. The methyl oleate content of the observed oils was 55.43% and 49.20%, respectively. Meanwhile, the methyl stearate content was 5.73% and 6.04%, respectively. It indicated that fatty acid in microalgae could be extracted into palm oil and then reacted with methanol in the presence of base catalyst (potassium hydroxide) to form methyl ester. Furthermore, generated methyl ester, known as the organic phase component, could be a co-solvent for algal oil extraction.

5.2 Comparison of two-stage method and simultaneous extraction transesterification

As shown in Figure 3, the composition of FAME for both methods was dominated by methyl oleate and methyl palmitate as major components. Methyl oleate percentage in the two-stage method was higher than that in the simultaneous extraction transesterification method in contrast to the methyl palmitate percentage. Meanwhile, the percentage of minor components, i.e., methyl myristate and methyl stearate, showed a slight difference in both methods. This result indicates that the unsaturated fatty acid component, represented by methyl oleate, in the product of the two-stage method was more dominant than that of simultaneous extraction transesterification. It was caused by the lower partial biodiesel yield from dry microalgae, as shown in Table 3, which had more saturated component than palm oil. Otherwise, the higher saturated component occurred in simultaneous extraction transesterification, which had more than twice of partial biodiesel yield from dry microalgae compared with the two-stage method. It should be noted that the unsaturated fatty acid component of biodiesel will reduce the stability of acid toward the oxidation process and decrease the cetane number of biodiesel from microalgae [35]. In other words, more

Table 3: Comparison of biodiesel yield in two-stage method and simultaneous extraction transesterification

Process method	Overall yield (%)	Yield _{palm} (%)	Yield _{dm} (%)
Two-stage method	50.69	61.28	3.69
Simultaneous extraction–transesterification	72.24	85.41	6.92

Figure 4: Major FAME composition for various methanol-palm oil molar ratios.

Table 4: Biodiesel yield for various methanol-palm oil molar ratios

Methanol-palm oil molar ratio	Overall yield (%)	Yield _{palm} (%)	Yield _{dm} (%)
8:1	64.08	76.05	5.09
10:1	72.24	85.41	6.92
12:1	68.76	81.30	6.37

Table 5: Biodiesel yield for various palm oil-microalgae weight ratios

Palm oil-microalgae weight ratio	Overall yield (%)	Yield _{palm} (%)	Yield _{dm} (%)
3:1	59.16	77.29	4.82
4:1	63.98	78.71	5.17
5:1	72.24	85.41	6.92
6:1	47.67	55.16	2.90

unsaturated fatty acid components in biodiesel will lower the quality of biodiesel as fuel.

Furthermore, Table 3 shows that simultaneous extraction transesterification has proven to generate more biodiesel than the conventional method, both

from palm oil and dry microalgae. In the conventional method, algal oil extraction was the limiting process as oil must be extracted from microalgae by using a solvent.

Figure 5: Major FAME composition for various palm oil-microalgae weight ratios.

Figure 6: Major FAME composition for various temperatures.

Table 6: Biodiesel yield for various temperatures

Temperature (°C)	Overall yield (%)	Yield _{palm} (%)	Yield _{dm} (%)
40	76.06	89.62	8.30
50	77.01	90.11	11.37
60	85.28	99.01	16.69

Afterward, the separation process would be conducted before oil undergoes transesterification to produce biodiesel. Hence, biodiesel yield was influenced by the effectiveness of palm oil as a solvent to extract the oil. Meanwhile, the higher yield of simultaneous extraction transesterification could be ascribed to the use of methanol as both a reactant for transesterification and a co-solvent for oil extraction. Moreover, as a product, biodiesel conducted as a co-solvent due to the similar organic phase to algal oil.

5.3 Effect of methanol–palm oil molar ratio

Figure 4 illustrates the composition of FAME for various methanol–palm oil molar ratios. Methyl oleate and methyl palmitate were dominant in biodiesel, so these two fatty acids were compared. For a methanol–palm oil molar ratio of 12:1, methyl oleate was increased dramatically and methyl palmitate was decreased. However, for the ratios of 8:1 and 10:1, there was no significant difference in methyl palmitate and methyl oleate percentage.

From Table 4, the highest yield was achieved by 10:1 ratio and decreased insignificantly when the molar ratio was increased to 12:1. Theoretically, increasing the molar ratio of reactants will increase the reaction rate. As transesterification is an equilibrium reaction, reactants must be added to shift the reaction to the product side. Stoichiometrically, transesterification reaction requires 3 moles of alcohol and 1 mole of triglyceride to yield 3 moles of ester and 1 mole of glycerol. However, the additional methanol not always corresponds to the increase in biodiesel yield because of extracted fatty acid limitation. Thus, the yields of biodiesel at ratios of 10:1 and 12:1 were slightly different.

5.4 Effect of palm oil–microalgae weight ratio

Figure 5 presents the composition of major component of FAME for various palm oil–microalgae weight ratios. Biodiesel composition for various palm oil–microalgae weight ratios shows a similar trend to the existing volume of biodiesel. Since the least weight of dry microalgae at ratio of 6:1 was used, it was therefore reasonable that its methyl palmitate content was the smallest. This tendency was inversely proportional to methyl oleate where the highest methyl oleate content was present in a ratio of 6:1. It could be attributed to methyl oleate, the main content of palm oil that is converted more into FAME. Adding microalgae in a ratio of 5:1 increased the methyl palmitate content and reduced the methyl oleate content as more methyl palmitates from microalgae were converted to biodiesel. As the quantity of microalgae increased in ratios of 4:1 and 3:1, the methyl

Table 7: Comparison of biodiesel yield from *Spirulina* Sp.

Previous findings	Process description	Biodiesel yield
This study	Single-stage simultaneous extraction–transesterification using palm oil–methanol mixture under alkaline conditions	16.69 wt% (w/w of biomass)
Shirazi et al. [29]	Direct transesterification near supercritical methanol conditions (12 MPa of pressure and 275°C of temperature)	<ul style="list-style-type: none"> • 99.32 wt% (w/w of lipid content) • Lipid content = 16 wt% (w/w of biomass)
Nautiyal et al. [39]	On the use of hexane–methanol mixture for single-stage extraction–transesterification under acid conditions	<ul style="list-style-type: none"> • 75 ± 0.4 wt% (w/w of lipid content) • Lipid content = 8.60 ± 0.20 wt% (w/w of biomass)
El-Shimi et al. [40]	Direct transesterification with H_2SO_4 catalyst (1 g/g lipid) at a reaction temperature of 65°C	9.3 wt% (w/w of biomass)
Sumprasit et al. [41]	Two-step process: <ul style="list-style-type: none"> - Lipid extraction by using osmotic shock with 1:2 chloroform/methanol mixture as a solvent - Conventional transesterification with H_2SO_4 catalyst (1 g/g lipid) 	7.1 wt% (w/w of biomass)
Pradana et al. [42]	Soxhlet extraction of algal oil using hexane as a solvent	Algal oil yield = 1.21 wt% (w/w of biomass)

palmitate content decreased until it was constant. The tendency was similar to the volume obtained. Microalgae addition caused the solution to be more viscous, making the removal of palmitate from microalgae and biodiesel conversion difficult.

Table 5 shows the overall biodiesel yield, the partial biodiesel yield from palm oil, and the partial biodiesel yield from dry microalgae for various palm oil–microalgae weight ratios. As more microalgae were used, the overall yield of biodiesel decreased. This indicated that the addition of overall biodiesel yield was derived from the conversion of extracted algal oil and palm oil during simultaneous extraction transesterification. Moreover, the increasing overall yield was always followed by increasing the partial biodiesel yield from palm oil and dry microalgae. It indicated that the biodiesel produced from palm oil supported as a co-solvent in extraction of algal oil to be converted into biodiesel.

The optimum biodiesel yield was achieved at a palm oil–microalgae weight ratio of 5:1. While biodiesel obtained decreased with the addition of microalgae for a ratio of 6:1, there was no significant difference in biodiesel production between 3:1 and 4:1 weight ratios. This was because when less microalgae were added, the viscosity of the solution was not excessively high so the reaction could still occur well enough and the algal oil could still be extracted as a reactant. However, after further microalgae addition, the viscosity of the solution became extremely high that the reaction rate decreased and yield of biodiesel yield reduced accordingly.

5.5 Effect of temperature

Biodiesel was obtained through simultaneous extraction transesterification of the mixture of palm oil–microalgae biomass using methanol as the esterification agent and potassium hydroxide as the catalyst. The reaction produced different results of fatty acid composition for various temperatures, which is shown in Figure 6. First, it is clear that the various temperatures did not greatly affect the composition of FAMES. At 40°C, FAMES consist of methyl palmitate (38%), oleate (45%), linoleate (12%), and stearate (5%). Meanwhile, at 50°C, the percentage of each methyl ester follows the prior temperature, except for methyl oleate with a slightly different amount of methyl oleate, 42%. Most noticeably, at 60°C there are significant results from the presence of methyl myristate (3%) and a scant decrease in methyl oleate percentage (41%), which is the lowest one. European standards EN14214 mentioned the requirements of biodiesel that it should meet the composition of linolenic acid (less than 12%) and polyunsaturated FA (>4 double bond), respectively [38]. In this experiment, microalgae biodiesel consisted of 12% linolenic acid, which has fulfilled the regulation and it makes the biodiesel feasible to be scaled up.

The result in Table 6 shows that the overall yield mounted up from 76% to 85% as the reaction temperature increased. Other than that, the increase in reaction temperature did not give an obvious impact on biodiesel yield. Therefore, 60°C was chosen as the optimum reaction temperature. This phenomenon occurred as the rate of reaction increased with the increase in temperature, causing

the occurrence of collisions between reactant molecules to be greater [36], as expressed by the Arrhenius equation. The transesterification reaction is classified as an exothermic reaction whose temperature increase precisely shifts the reaction balance toward the reactant. As a result, the number of products and the conversion decrease when temperature increases. However, the increase in temperature in this work was limited by low boiling point of methanol (64.7°C). The increase in temperature of more than 60°C will drive methanol in the form of gas, causing less contact with oil to produce FAME [37]. Therefore, the reaction temperature is preferably chosen below the boiling point of methanol. Leung and Guo [37] and Sharma et al. [38] reported the same result on the FAME yield. Based on their experiment that 60°C was found as the optimum temperature with the biodiesel yield were 84.08% and 88.80%, consecutively.

5.6 Comparison of previous study

Table 7 shows some previous research studies, which have been conducted using *Spirulina* sp. through several processes. As conventional simultaneous extraction transesterification is still hampered by low yield results, it is fully recommended to use the mixture of palm oil and microalgae to attain the higher overall yield (85.28%) than merely employing microalgae.

The increase in temperature under single-stage simultaneous extraction–transesterification resulted in the positive trend of overall and partial biodiesel yields. The highest biodiesel yield from dry microalgae was 16.69 wt% (85.28 wt% of overall yield), obtained at a temperature of 60°C. Compared to previous findings, this method improved the effectiveness of biodiesel production from *Spirulina* sp.

Acknowledgments: The authors would like to thank the Ministry of Education and Culture of the Republic of Indonesia and Universitas Gadjah Mada, Indonesia for financial support.

Conflict of interest: The authors declare no conflict of interest.

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