Extractive-transesterification of Microalgae Arthrospira sp. Using Methanol-Hexane Mixture as Solvent

Yano Surya Pradana*‡, Wildan Masruri*, Fariz Azwar Azmi*, Eko Agus Suyono**, Hanifrahmawan Sudibyo*, Rochmadi*

* Chemical Engineering Department, Faculty of Engineering, Universitas Gadjah Mada, Jalan Grafika No. 2, Yogyakarta, 55281, Indonesia

** Faculty of Biology, Universitas Gadjah Mada, Jalan Teknika Selatan, Sekip Utara, Yogyakarta 55281, Indonesia

(yanopradana@ugm.ac.id, wildan.masruri@mail.ugm.ac.id, fariz.azwar.a@mail.ugm.ac.id, eko_suyono@ugm.ac.id, hanifrahmawan.s@mail.ugm.ac.id, rochmadi@ugm.ac.id)

‡Corresponding Author; Yano Surya Pradana, Chemical Engineering Department, Faculty of Engineering, Universitas Gadjah Mada, Jalan Grafika No. 2, Yogyakarta, 55281, Indonesia, Tel: +62 274 649 2171,
Fax: +62 274 649 2170, yanopradana@ugm.ac.id

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Abstract- Recently, the concern of energy crisis and global warming threat has driven the development of renewable and sustainable fuels production. One of the considered options of renewable fuels is biodiesel synthesized from microalgae. To produce biodiesel from microalgae, the extractive-transesterification process is used to extract and convert algal oil into biodiesel in a single step process, simplifying the process and lowering energy requirement. In this study, experiments were conducted in a batch stirred reactor to investigate the comparison of alkaline (sodium hydroxide) versus acid (sulfuric acid) catalyst and the effect of biomass weight-solvent volume ratio (1:3, 1:5, 1:7) on biodiesel yield from microalgae *Arthrospira sp*. A methanol-hexane mixture was used as a solvent at temperature of 50ºC and methanol-hexane volumetric ratio of 3:7. The highest overall biodiesel yield was 13.85% w/w of dry biomass, obtained at a ratio of 1:5 using an alkaline catalyst. Compared to the previous studies, extractive-transesterification using methanol-hexane mixture as solvent improved the biodiesel yield from microalgae.

Keywords Biodiesel, microalgae, *Arthrospira sp.*, extractive-transesterification, methanol-hexane mixture.

1. Introduction

In the last ten years, the concern for renewable and sustainable energy requirement has been increasing along with the fast depletion and the uncertain fossil fuel price [1-4]. Moreover, the reserves of fossil fuels are predicted to decrease significantly in the next decades, threatening world's energy security [5]. It has pushed all academician to find renewable resources that should be safe for human and economically competitive [6]. Then, biofuels appear as a promising option of renewable energy resources to solve these issues [7]. They are also expected to decrease the heavy consumption rate of conventional fuels which affects the global climate change [8].

One of the considered biofuels is biodiesel. This bio-based fuel is projected to substitute the dominant use of petrol-diesel in diesel engine [9]. It is supported from experimental data that biodiesel has similar properties with conventional diesel fuel, i.e. lubricity, cetane value and flash point [10], hence, engine modification is not or little required in using biodiesel. Moreover, the application of biodiesel has several advantages in pure or blended with petrol-diesel: (i) lower emission; (ii) easier to degrade; and (iii) less toxic [11-13]. Thus, the use of biodiesel also complies with significant mechanical aspects in automotive manufacturers [14].

Biodiesel is mainly synthesized by transesterification of triglycerides from various feedstocks with short-chain

alcohols, such as methanol and ethanol, in the presence of catalyst [15,16]. Several biodiesel feedstocks usually come from oil crops produced in large-scale capacities, such as soybean oil [17], palm oil [18], and sunflower oil [19]. However, due to the edible oil, the supply of these raw materials in the energy sector is potentially conflicting with food sector [20,21]. Also, the use of these raw materials for biodiesel production also requires large plantation area for maintaining their availability, causing massive land provision and ecosystem damage [22].

As an effort on improving economic aspect, low-cost materials are promoted to generate biodiesel, i.e. jatropha oil [23], nyamplung (*Callophyllum inophyllum*) oil [24], waste cooking oil [25,26] and fatty acid distillate from palm oil refining process [27]. In addition, the utilization of these materials will reduce wastes and encourage the production of non-edible oils to be converted into higher value products [28]. However, the impurity components are still a problem in the product. Recently in the past few years, microalgae have attracted many interests to be explored further as biodiesel feedstock [29,30].

Microalgae are a micro-photosynthetic organism that can be cultivated in a wide range of growth medium conditions, including the extreme medium such as industrial wastewater [31]. Microalgae cell converts carbon dioxide and water using lights into carbohydrates (cellulose), proteins, lipid and other compounds, which can be applied as smart food, feed and energy [32,33]. Compared to oil crops, these microorganisms have some advantages: (i) faster growth rate; (ii) higher biomass productivity; (iii) higher effectivity on capturing and storing CO_2 emission [34-36]. However, the industrialization of microalgae-based biodiesel production still faces obstacles, mainly on production cost [37].

The bottleneck of the process is caused by the ineffective of the sequential two-step process of oil extraction and transesterification, well-known as conventional process [38]. The oil extraction step includes cell wall disruption, after which the solvent can diffuse into inner cell and extract oil out of the cell [39]. After separated from solid residue and solvent, extracted algal oil is then continued by transesterification, converting it into methyl ester [40]. The evaluation shows that this conventional process requires more energy and promotes inter-process complexity [41]. To overcome this bottleneck, the single step process, called extractive-transesterification process, is suggested for improving the effectivity of microalgae-based biodiesel synthesis.

Extractive-transesterification is a process of directly transforming biomass-derived fatty acid into biodiesel by combining extraction and transesterification in a single step [42]. Hypothetically, this method can reduce oil loss in inter-process stage, yielding more biodiesel than conventional method [43]. To conduct the extractive-transesterification, solvent, in the mixture form of alcohol and other chemicals, is required to be mixed with microalgae in one reactor. This study proposed the mixture of methanol and hexane to carry out the process. As solvent and reactant, methanol was mixed with hexane to optimize the fatty acid extraction.

This paper presented extractive-transesterification as an advanced process of biodiesel production from microalgae *Arthrospira sp.* using methanol-hexane mixture as solvent. *Arthrospira sp.* is classified as high biomass productivity microalgae that are promising in a sustainable supply for application of energy-efficient algae refinery concept proposed by Jamilatun et al. [44]. Despite its low oil content for biodiesel production, the biomass residue can still be converted further into other energy products, i.e. bio-oil and syngas. The comparison of alkaline (solid sodium hydroxide) vs acid (sulfuric acid) catalyst and the effect of biomass (*Arthrospira sp.*) weight-solvent volume ratio on biodiesel yield were investigated. Moreover, the composition of fatty acid methyl esters in biodiesel was also discussed and compared to the previous study.

2. Materials and Methods

2.1. Materials

Fresh dry microalgae *Arthrospira sp.*, as applied in the study conducted by Jamilatun et al. [44], was supplied from Lamongan, Indonesia. *Arthrospira sp.* was cultivated in modified Walne's Medium and open pond system, under light intensity of 12,000-15,000 lux, medium temperature of 25-30^{o}C, pH of 7-8 and salinity of 0.5-1.5%. This biomass was characterized by using chemical composition, proximate, and elemental analysis as shown in Table 1. The chemical composition analysis results depict the low lipid content (0.25%), high carbohydrate (46.13%) and protein content (44.72%). The volatile matter composition (67.03%), O/C (0.59) and H/C molar ratio (1.95) presented in promising value for generating energy, reflected on higher heating value (HHV) of 20.09 MJ/kg. Moreover, the small O/C molar ratio revealed the low polar compounds, as undesired fuel content. Meanwhile, the H/C molar ratio also represented the prominent composition of straight-chain hydrocarbon.

The other raw materials used in this study were methanol, n-hexane, sodium hydroxide, sulfuric acid, and distilled water. Methanol (≥99.8%) was supplied from Petronas Methanol (Labuan) SDN BHD. N-hexane (≥96.0%), sodium hydroxide (≥97.0%) and sulfuric acid (≥98%) were manufactured by Merck. Meanwhile, distilled water was produced by UD. Jaya Santosa, Yogyakarta, Indonesia.

2.2. Methods

2.2.1. Conventional Two-step Process

The conventional method of converting microalgae *Arthrospira sp.* into biodiesel was conducted in two-step batch process, namely extraction and transesterification. Each of batch extractor and reactor, in a different unit, used in this study was similar with equipment set arranged by Pradana et al. [45]. It consisted of a three-necked round-bottom flask with a reflux system, equipped with heating mantle, thermometer and mechanical stirrer, as presented in Fig. 1. At the initial time, 500 mL of hexane were inserted

into extractor for the heating process. After reaching 50°C of temperature, 150 g of dry microalgae was fed into the reactor and stirred at stirring rate of 600 rpm. This process was carried out for two hours. After the extraction was completed, the mixture was separated into liquid phase and solid residue by vacuum filtration. The liquid phase was then heated at atmospheric pressure to remove hexane in a set of purification unit, as shown in Fig. 2. The remaining component after hexane removal was algal oil. Algal oil weight was then measured.

Table 1. Analysis results of microalgae *Arthrospira sp.* as biodiesel feedstocks [44]

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

Algal oil and methoxy solution (50 mL of methanol and 1% w/w by algal oil weight of sodium hydroxide) were poured into the reactor for transesterification process section using the same equipment arrangement in Fig. 1. The reaction was conducted for two hours. After the reaction was completed, the mixture was then washed with 50°C distilled water and decanted to separate biodiesel in the upper layer and water-phase liquid in the lower layer. The remaining water in the upper layer liquid was then removed by heating it in a set of purification unit (Fig. 2). In the end, the dark liquid was obtained as biodiesel which will later be analysed.

2.2.2. Extractive-transesterification

Extractive-transesterification of microalgae *Arthrospira sp.* was conducted in a batch-system reactor as illustrated in Fig. 1. At the initial time, methanol-hexane mixture in the volumetric ratio of 3:7 and biomass weight-solvent volume ratio (1:3, 1:5 and 1:7) were fed into the reactor for the heating process with stirring rate of 600 rpm. This was done using 0.15 g of solid sodium hydroxide as alkaline catalyst and 4.1 mL of sulfuric acid as acid catalyst. After reaching 50°C of temperature, 150 g of dry microalgae was fed into the reactor. The process was carried out for two hours.

After completing the reaction, the mixture was separated into a liquid phase and solid residue by vacuum filtration. The liquid phase was then washed with 50°C distilled water and decanted to separate biodiesel in the upper layer and water-phase liquid in the lower layer. The purification step for the remaining water, methanol and hexane was conducted with the same procedure in the conventional method. Obtained biodiesel was then analysed as described hereinafter.

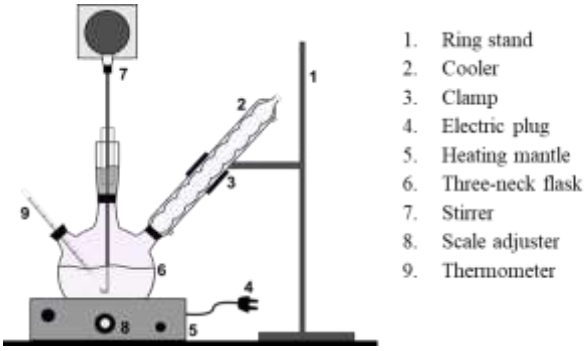


Fig. 1. Equipment set for extractive-transesterification [45].

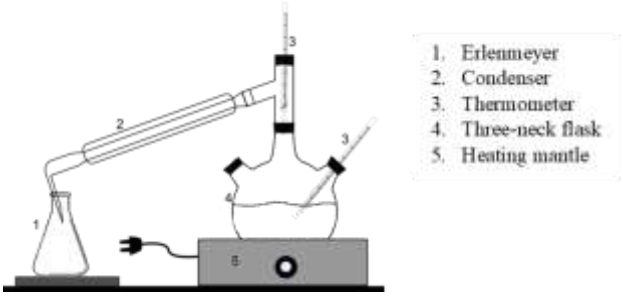


Fig. 2. Equipment set for washed-biodiesel purification.

2.2.3. Product analysis

The yield of biodiesel from microalgae was determined by measuring the total mass of biodiesel obtained from the process. Then, fatty acid methyl esters (FAMEs) composition of biodiesel was analysed by using Gas Chromatography-Mass Spectrometry (GC-MS). The GC-MS used in this analysis was SHIMADZU QP2010S with a column type of AGILENT HP-5, inner column diameter of 0.25 mm, column length of 30 m and helium as the carrier gas. Before analysis, samples of biodiesel were dissolved using n-hexane. The sample was then injected in GC-MS with split injection mode using a split ratio of 139.0 and injection temperature of 300°C. The pressure of the column was programmed at 13.0 kPa. The temperature of GC-MS column oven was set at 50°C for holding time of 5 min. The heating rate was

measured at 5°C.min^{-1} up to 280°C, and then it was maintained for 19 min.

2.2.4. Data analysis

The biodiesel yield determined in this study was calculated by comparing biodiesel weight (m_{bio}) to initial dry biomass (m_{db}), as shown in Equation (1).

$$\text{Yield (\%)} = m_{bio}/m_{db} \times 100\% \quad (1)$$

3. Results and Discussion

3.1. Conventional Two-step Process

The conventional process of biodiesel production from microalgae *Arthrospira sp.* was carried out in two processes of extraction and transesterification, separately. It resulted in 3.58±0.2% of biodiesel yield. The result of the FAME components analysis of the biodiesel using GC-MS analysis is presented in Fig. 3. The summary of GC-MS result was depicted in Fig. 4. It shows that methyl palmitate and oleate were the two main methyl esters present in the product, with a percentage of 40.58% and 44.60%, respectively. The impurities or other components were not more than 1.10%.

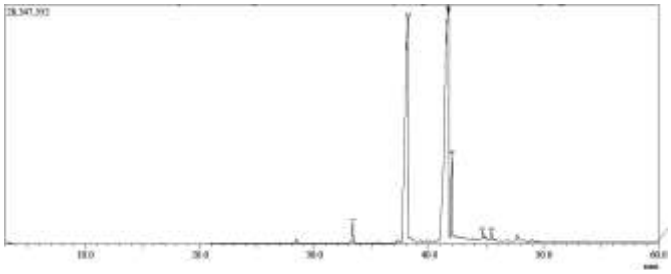


Fig. 3. The GC-MS result of biodiesel from *Arthrospira sp.* using alkaline catalyzed conventional two-step process.

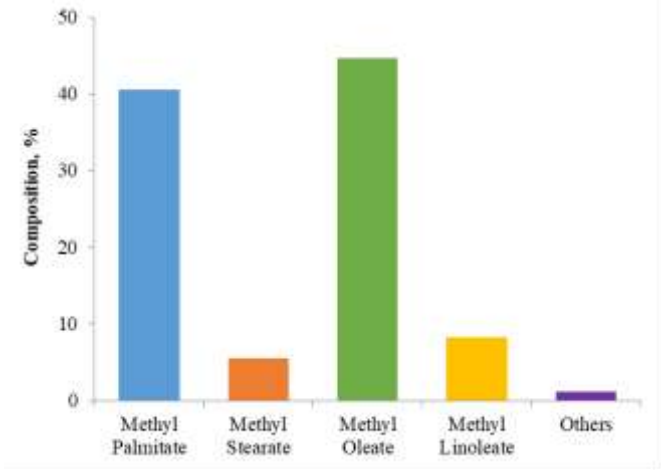


Fig. 4. FAME composition of biodiesel from *Arthrospira sp.* using alkaline catalyzed conventional two-step process.

3.2. Extractive-transesterification

3.2.1. Alkaline vs Acid Catalyst

Fig. 5 shows the comparison of biodiesel mass and yield of extractive-transesterification from microalgae *Arthrospira sp.* at temperature of 50°C. The catalysts compared to this study were sodium hydroxide as alkaline catalyst and sulfuric acid as acid catalyst. The process using alkaline catalyst produced biodiesel from microalgae with a yield of 11.68% w/w of dry biomass. Meanwhile, the acid catalysts showed a significantly slower extractive reaction, resulting in a lower biodiesel yield of 0.42% w/w. Sodium hydroxide catalyst, together with methanol-hexane mixture, forced the reaction rate of extractive-transesterification into biodiesel to go faster than acid catalyst.

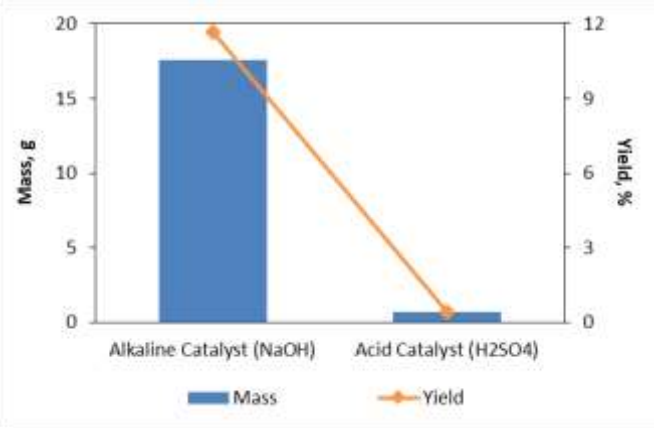


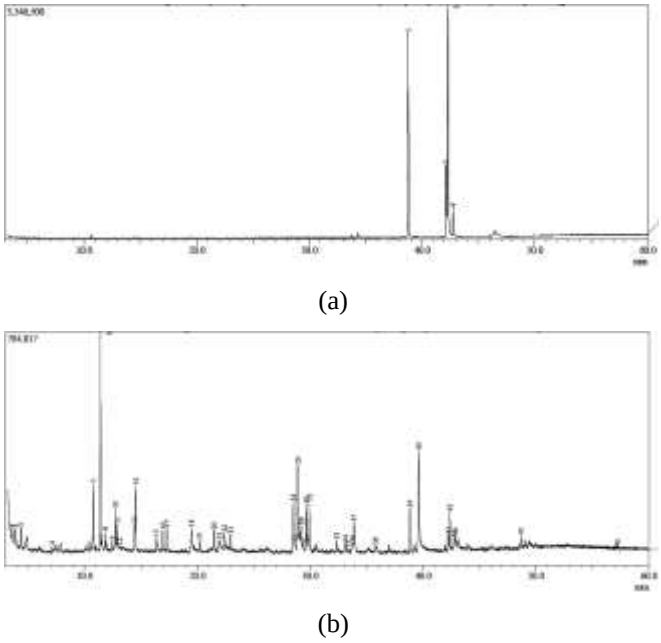
Fig. 5. Comparison of biodiesel mass and yield in using alkaline and acid catalyzed extractive-transesterification.

Fig. 6 illustrates the results of GC-MS of biodiesel from microalgae *Arthrospira sp.* catalyzed by sodium hydroxide (a) and sulfuric acid (b) at temperature of 50°C. Meanwhile, the comparison of FAME composition from GC-MS analysis using alkaline and acid catalyst is shown in Fig. 7. The composition of biodiesel synthesized using alkaline catalyst was dominated by methyl palmitate (54.00%), followed by methyl linoleate (34.03%) and methyl stearate (11.96%). Furthermore, the biodiesel obtained from the reaction using acid catalyst had components of methyl palmitate, methyl stearate, methyl linoleate, methyl oleate and others. The low biodiesel yield of acid catalyzed extractive-transesterification has an effect on the less effectivity of fatty acid methyl esters extraction, represented by the high percentage of others component (27.42%). Thus, the optimum extractive-transesterification of microalgae *Arthrospira sp.* using methanol-hexane solvent was achieved at alkaline condition.

3.2.2. Effect of Biomass Weight-Solvent Volume Ratio on Biodiesel Yield

The effect of biomass weight-solvent volume ratio on biodiesel yield of extractive-transesterification from microalgae *Arthrospira sp.*, catalysed by sodium hydroxide at temperature of 50°C, is presented in Fig. 8. The variation of biomass weight-solvent volume ratio studied was 1:3, 1:5 and 1:7. The results denoted the significant increase of

biodiesel yield at ratio of 1:5 (13.85%), compared to the ratio of 1:3 (3.84%). This result was supported by extraction and transesterification process theory: more solvent led the more oil extracted from algae cell. Since methanol also acted as a reactant for transesterification, it promoted the conversion of algae oil into biodiesel. However, the ratio of 1:7 resulted in a lower yield than ratio of 1:5. This phenomenon was attributed to the soap formation as by-product. The saponification reaction occurred because the reaction rate between the fatty acid in free condition with alkali was very fast or even spontaneous. Thus, the optimum biomass weight-solvent ratio obtained was 1:5.



(a)

(b)

Fig. 6. The GC-MS result of biodiesel from *Arthrospira sp.* using: (a) alkaline (sodium hydroxide); (b) acid (sulfuric acid) catalyzed extractive-transesterification.

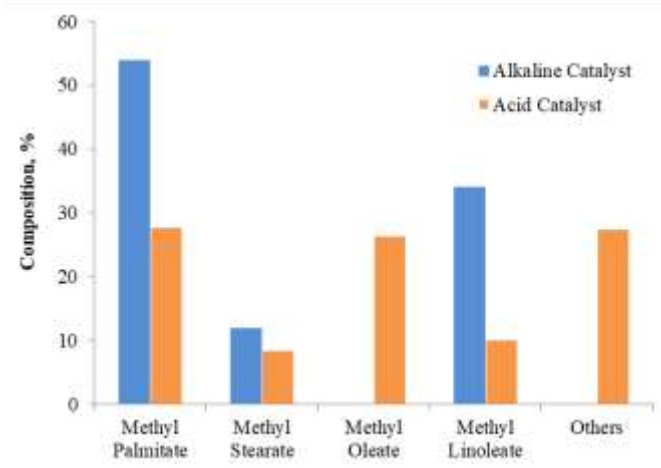


Fig. 7. Comparison of FAME composition of biodiesel in using alkaline and acid catalyzed extractive-transesterification.

Fig. 9 presents the summary of FAME composition of biodiesel on various biomass weight-solvent volume ratio. Methyl palmitate and linoleate component were still dominant in all biodiesel samples. The low biodiesel yield at a ratio of 1:3 is attributed to the fatty acid component which was extracted and converted into FAME, consisted of methyl palmitate and linoleate. The composition of FAME was different at ratio of 1:5 and 1:7 which also contains methyl palmitate, linoleate and oleate in various percentage. Moreover, for the ratio of 1:7, other components are present in significant content (22.35%) which was related to the soap formation.

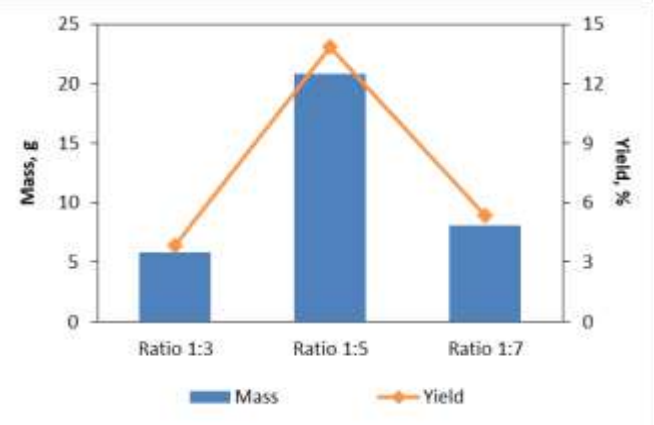


Fig. 8. Comparison of biodiesel mass and yield on various biomass weight-solvent volume ratios.

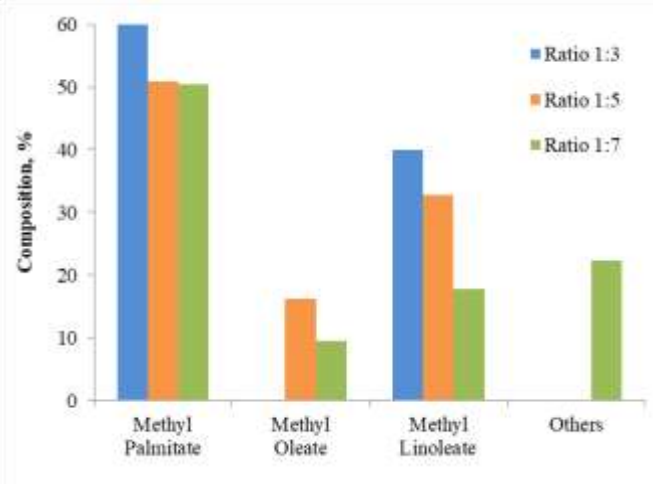


Fig. 9. FAME composition of biodiesel on various biomass weight-solvent volume ratios.

3.2.3. Remaining Components in Microalgae Solid Residue

Table 2 shows the analysis results of the remaining components of solid residue as by-product in extractive-transesterification of microalgae *Arthrospira sp.* using alkaline catalyst. The lipid content of solid residue was 0.09%, which is significantly smaller than in the fresh *Arthrospira sp.* shown in Table 1 (0.25%). This indicates that extractive-transesterification used almost all algal oil out from the cell, but small changes were observed for carbohydrate, protein and volatile content. For instance, the volatile matter of this residue (68.31%) was still similar with fresh biomass (67.03%). However, the low O/C molar ratio (0.64) illustrated a small content of oxygenates, which was the unwanted compounds. Meanwhile, the high H/C molar ratio (1.91) showed the dominant composition of aliphatic

hydrocarbon content. Based on these values, the residue of microalgae still has a promising heating value [45]. It was reflected on higher heating value (HHV) of 18.21 MJ/kg, only slightly lower than that of fresh *Arthrospira sp.* (20.09 MJ/kg).

Table 2. Analysis results of microalgae A*rthrospira sp.* solid residue as a by-product [44]

Component	Composition
Chemical composition analysis (%)	
Carbohydrate	38.51
Protein	49.60
Lipid	0.09
Proximate analysis (%)	
Volatiles	68.31
Fixed carbon	12.77
Moisture	9.99
Ash	8.93
Elemental analysis (%)	
Carbon	41.36
Hydrogen	6.60
Oxygen	35.33
Nitrogen	7.17
Sulphur	0.55
H/C, molar ratio	1.91
O/C, molar ratio	0.64
Ca	1.25
Mg	0.48
K	0.51
Higher heating value (MJ/kg)	18.21

3.3. Comparison with Other Results in Previous Studies

The highest biodiesel yield was 13.85% w/w of dry biomass, obtained at ratio of 1:5 using alkaline catalyst. This result was then compared to previous studies, as presented in Table 3. It was proved that extractive-transesterification using methanol-hexane mixture as solvent improved the biodiesel yield from microalgae. In addition, the comparison of FAMEs composition produced from microalgae *Arthrospira sp.* in several studies is shown in Table 4. Generally, the component of FAME from *Arthrospira sp.* consisted of C12-C18 in various percentages. The highest content of biodiesel in all studies was mentioned as palmitic methyl ester, except Liu et al. [46] which reported two dominant components of myristic and linoleic methyl ester in their obtained biodiesel using sulfuric acid-methanol mixture.

4. Conclusion

The results of extractive-transesterification of *Arthrospira sp.* showed that the highest biodiesel yield was 13.85% w/w of dry biomass, obtained at biomass weight-solvent volume ratio of 1:5 using alkaline (sodium hydroxide) catalyst. Compared to the previous studies, extractive-transesterification using methanol-hexane mixture as solvent improved the biodiesel yield from microalgae.

Acknowledgements

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Table 3. Comparison of biodiesel yield obtained from *Arthrospira sp.*

Research	Process	Biodiesel yield
This study	Sodium hydroxide catalyzed extractive-transesterification using methanol-hexane mixture as solvent	13.85% w/w of dry biomass
Nautiyal et al. [3]	Single stage extraction-transesterification using hexane-methanol mixture and acid catalyst	6.45±0.18% w/w of dry biomass
Shirazi et al. [6]	One-step extraction transesterification process in sub/supercritical methanol condition (T = 275°C and P = 12 MPa)	15.89% w/w of dry biomass
Liu et al. [46]	Direct transesterification using H_2SO_4-methanol mixture in batch reactor	0.26±0.02% w/w of dry biomass (2.6±0.2 µg/mg dry biomass)
El-Shimi et al. [47]	H_2SO_4 catalyzed direct transesterification using at temperature of 65°C	9.3% w/w of dry biomass
Sumprasit et al. [48]	Two-step process: oil extraction using chloroform-methanol mixture and oil transesterification using H_2SO_4 as acid catalyst	7.1% w/w of dry biomass
Pradana et al. [29]	Oil recovery using n-hexane extraction in soxhlet apparatus	Algal oil yield = 1.21% w/w of dry biomass

Table 4. Comparison of fatty acid methyl ester profiles of biodiesel obtained from *Arthrospira sp.*

Fatty Acid Methyl Ester	Composition (%)					
	This study			Liu et al. [46]	El-Shimi et al. [47]	Sumprasit et al. [48]
	Conventional two-step process using alkaline catalyst	Extractive-transesterification				
		Alkaline catalyst	Acid catalyst			
Lauric (C12:0)	-	-	-	-	0.7	-
Myristic (C14:0)	-	-	-	16.2±0.1	20.9	9.73
Palmitic (C16:0)	40.58	54.00	27.72	11.0±0.2	48.35	39.19
Palmitoleic (C16:1)	-	-	-	8.3±0.2	2.66	6.96
Stearic (C18:0)	5.48	11.96	8.42	-	2.02	0.37
Oleic (C18:1)	44.60	-	26.35	9.6±0.1	2.41	38.97
Linoleic (C18:2)	8.25	34.03	10.09	9.6±0.2	5.37	4.78
Linoleuic (C18:3)	-	-	-	15.5±0.4	7.84	-

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