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Fatty Acid of Microalgae as a Potential Feedstock for Biodiesel Production in Indonesia

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Abstract. Demand for fossil fuel of coal, petroleum and natural gas has always increased from year to year. Therefore, the development, expansion and utilization of biomass from non fossil fuels need be carried out. Efforts to explore, develop, process and utilize biomass from non-fossil fuels, which is the cultivation of microalgae containing triglycerides that can be extracted into methyl esters (biodiesel). The sample extraction process is carried out in 5 stages, such as harvesting, weaving, methylation, extraction, and washing. The amount and ratio of saturated and unsaturated fatty acid is a key that determines the suitability of microalgae as a biofuel feedstock. This paper aims to compare the potential microalgae in Indonesia with the other countries as feedstock for biofuel production. The fatty acid composition of *Synechococcus sp.* HS-9, *Spirulina platensis*, *Glagah consortium microalgae*, and *Nostoc HS-20* from Indonesia could be a promising feedstock for biofuel production. The quantity of fatty acid microalgae from Indonesia higher than the other countries.

INTRODUCTION

Demand for fossil fuel of coal, petroleum and natural gas has always increased from year to year. Fossil fuels are natural resources that cannot be renewed and their reserves are depleting. Its fully realized that today there is climate change, rising fuel demand, and continuous exploitation of limited petroleum resources, which raise the problem of national fuel and energy needs. Therefore, the development, expansion and utilization of biomass from non fossil fuels need be carried out [1]. Efforts to explore, develop, process and utilize biomass from non-fossil fuels, which is the cultivation of microalgae containing triglycerides that can be extracted into methyl esters (biodiesel). To improve process efficiency, by-products such as biomass residues can be fermented to produce biogas which is used as a fuel generator that generates electricity [2]. This is an appropriate technology approach with low and efficient costs.

Microalgae has attracted attention to produce bioenergy because they can produce oil in the body's cells. The content of oil in microalga ranges from 20-50 % and microalgae can exceed 80 % of the weight of dry biomass [3]. Based on the criteria used to choose microalgae as a suitable raw material for biofuel production, each microalgae species produces different lipid, protein and carbohydrate ratios. Chemical composition of biofuel source microalgae has been studied previously, for example *Chlorella protothecoides* has a percentage of lipids (55 %), proteins (10-52 %) and carbohydrate (10-15 %) [4]. *Chlorella vulgaris* has percentages of lipids (14-22 %), proteins (51-58 %) and carbohydrate (12-17 %) [5]. GC analysis of fatty acid methyl esters of the selected cyanobacterial strain (*Synechococcus sp.* HS01) grown in media supplemented with ostrich oil showed a high content of C16 (palmitoleic acid and palmitic acid) and C18 (linoleic acid, oleic acid and linolenic acid) fatty acids of 42.7 and 42.8 %, respectively [6]. Some microalga can also synthesize a large quantity of polyunsaturated fatty acid such as C22:6 (42 %) in *Aurantiochytrium sp.*, C22:5 + C22:6 (39.4 %) in *Schizochytrium limacinum*, and C20:5 (25 %) in *Porphyridium cruentum* [7].

Indonesia and the other countries as tropical nation have a diversity of crops and microalgae which are potential for biodiesel. Tabel 1 compares oil yields of microalgae with other oil feedstocks [8]. It can be seen from the significant difference in biomass productivity, oil yield and biodiesel productivity between microalgae and the other crops. Microalgae have higher biomass productivity, oil and biodiesel yield compared to others. This paper aims to compare the potential microalgae in Indonesia with the other countries as feedstock for biofuel production.

TABLE 1. Comparison of microalgae with other biodiesel feedstocks [8]

Oil feedstocks	Oil content	Oil yield	Land use	Biodiesel productivity
	(% dry wt. Biomass)	(L oil/ha/year)	(m ² /year/L biodiesel)	(L biodiesel /ha/year)
Oil Seeds				
Microalgae (low oil content)	30	58.700	0,2	61.091
Microalgae (medium oil content)	50	97.800	0,1	101.782
Microalgae (high oil content)	70	136.900	0,1	142.475
Corn (<i>Zea mays</i>)	44	172	56	179
Soybean (<i>Glycine max</i>)	18	636	15	661
Sunflower (<i>Helianthus annuus</i>)	40	1.070	9	1.113
Caster (<i>Ricinus communis</i>)	40	1.307	8	1.360
Palm oil (<i>Elaeis guineensis</i>)	36	5.366	2	5.585

CULTIVATION OF MICROALGAE

The microalgae cultivation method significantly influences growth characteristics, yield, and composition [9]. Microalgae is autotropic organisms that can carry out photosynthesis. Microalgae requires light, CO₂, water and organic nutrition for photosynthesis process. Microalgae can be cultivated by different methods such as closed culture systems as though photo bioreactors (PBRs) or open ponds that refer to raceway pond and hybrid system [10]. The cultivation of microalgae using photo bioreactors is one of the most important factors for photosynthesis and breeding [11]. Photo bioreactors have an important role to minimize the risk of contamination, improve the reproducibility of the culture conditions, control hydrodynamic and temperature conditions, and also enable appropriate technical design [12].

EXTRACTION OF FATTY ACID

The sample extraction process is carried out in 5 stages, such as harvesting, weaving, methylation, extraction, and washing and shown in Figure 1 [13].

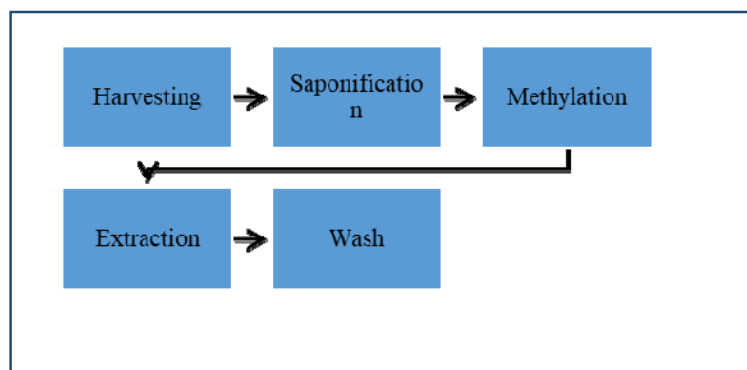


FIGURE. 1. The fatty acid extract preparation procedures

LIPID COMPOSITION OF MICROALGAE

Lipids located inside microalgae cells can generally be divided into two large subclasses based on the polarity of the molecular head groups: polar lipid and neutral lipids [14]. Polar lipid mainly contain a high quantity of Poly Unsaturated Fatty Acids (PUFAs). Storage lipids are mainly in the form of triglycerides (TAGs), having a high content of saturated FAs and some unsaturated FAs [7]. Neutral lipids for energy storages consist of acylglycerols and free fatty acids, which have fatty acyl groups and a hydrogen atom attached to the glycerol backbone, respectively [14]. The amount and ratio of saturated and unsaturated fatty acid is a key that determines the suitability of microalgae as a biofuel feedstock. Generally, unsaturated fatty acids, especially palmitoleic (16:1), oleic (18:1), linoleic (18:2), linolenic acid (18:3) and the saturated fatty acids of palmitic (16:0) are the main compositions of the oil generated by microalgae with only a small proportion made from saturated acids of stearic (18:0) [7].

FATTY ACID ANALYSIS

The results of fatty acid analysis of microalgae from the nine countries in the world showed as follows. The fatty acid analysis which observed from 4 strains In Indonesia and India. The strains *Synechococcus sp.* HS-9 isolated from Rawa Danau Banten, Indonesia and cultured at Laboratory of Plant Taxonomy, Department of Biology, Universitas Indonesia. It had saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) 7.58 %. From the analysis of the fatty acids obtained, results illustrated that the unicellular cyanobacteria strains (*Synechococcus sp.* HS-9) had a total polyunsaturated fatty acid (PUFA) 0 %, branched fatty acids 19.64 % and hydroxyl-substituted 12.08 % [13]. *Spirulina platensis* is a marine microalga which used in this investigation was obtained from The Research Institute for Marine Fisheries at Lampung in Indonesia. It had high amount of SFA (81.99 ± 3.91 %), low MUFA (9.45 ± 1.07 %) and PUFA (7.39 ± 0.66 %) [15]. *Glagah consortium microalgae* is microalgae have been isolated from Glagah in the Special Region of Yogyakarta Province, Indonesia. It had high amount of SFA 39.97 %, MUFA 33.42 % and PUFA 11.8% [16]. Microalgae, *Chlorella marina*, were isolated from Cochin Estuary, a tropical ecosystem, South west coast of India. In *Chlorella marina*, the major fatty acids like SFA, MUFA and PUFA were estimated to be in the range of 44.8 %, 24.01 % and 32 % of the total fatty acid content respectively [17].

Other fatty acid analysis which produced from 4 strains in Malaysia, Mexico, Portugal and Brazil. Microalgae such as *Botryococcus sp.* was isolated from TNJER (Taman Negara Johor Endau-Rompin) located in the southern region of Peninsular Malaysia. This study found that the percentages of SFA, MUFA and PUFA were 38.7 %, 16.7 % and 44.9 % respectively [18]. *Aphanocapsa marina*, microalgae species isolated from the Baja California Peninsula-Mexico, had a high conten (53 %) of SFA and (47 %) of MUFA [19]. *Desmochloris sp.* was isolated from the Red Sea and cultivated in Center of Marine Science, University of Algarve-Portugal. It also presented SFA, MUFA and PUFA, representing 39.7 %, 28.1 % and 32 % of the total fatty acids respectively [20]. *Isochrysis galbana* cultured at the Department of Food Science, Federal University of Santa Catarina-Brazil. The SFA, MUFA and PUFA content of total fatty acids were calculated and are expressed as 58 %, 28 % and 12.5 % respectively [21].

On the other hand, fatty acid analysis which generated from 3 strains in China, Indonesia and USA-Brazil. *Monoraphidium sp.* KMN5, strain of microalgae, was isolated at Lake Ming in Daqing, Heilongjiang Province, China. The lipid of *Monoraphidium sp.* KMN5 was composed of 58.7 % SFA, 11.7 % MUFA and 4.56 % PUFA [22]. *Nostoc HS-20* was cultured at Laboratory of Plant Taxonomy, Department of Biology, FMIPA University of Indonesia and was found the percentages of SFA 50.5 %, MUFA 23.4 % and PUFA 9.7 % [13]. The last strain of microalgae, *Botryococcus braunii*, was acquired by the the UTEX culture collection (The Culture collection of Algae, University of Texas at Austin) and cultured in the Microalgae Bank of the Marine Biology Lab (LABIOMAR) of the Federal University of Bahia (Brazil). It had SFA 55.25 %, MUFA 34.4 % and PUFA 10.3 % [23]. Figure 2 showed the comparison of each type of the total fatty acid from 11 strains of microalgae in 9 countries.

According to the fatty acid composition of *Synechococcus sp.* HS-9, *Spirulina platensis*, *Glagah consortium microalgae*, and *Nostoc HS-20* from Indonesia, these strain could be a promising feedstock for biofuel production. The quantity of fatty acid microalgae from Indonesia higher than the other countries. Therefore, the ability of microalgae to produce a high quantity of lipid and high quality of the fatty acid composition must be considered while looking for efficient microalgae strains for producing biofuels. In addition, most microalgae species can thrive under extreme conditions, in which their growth rate, lipid content and fatty acid composition are significantly affected. Understanding the effects of such situations on microalgae behavior gives us a controlling parameter to achieve more favorable results.

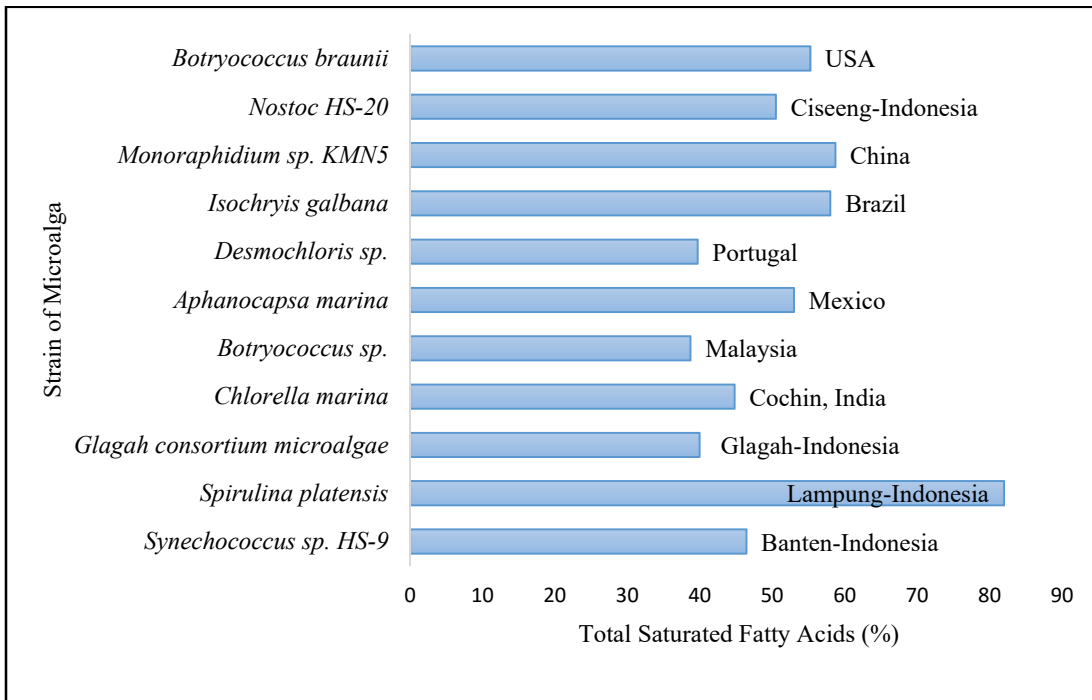


FIGURE. 2a. Total saturated fatty acid (SFA)

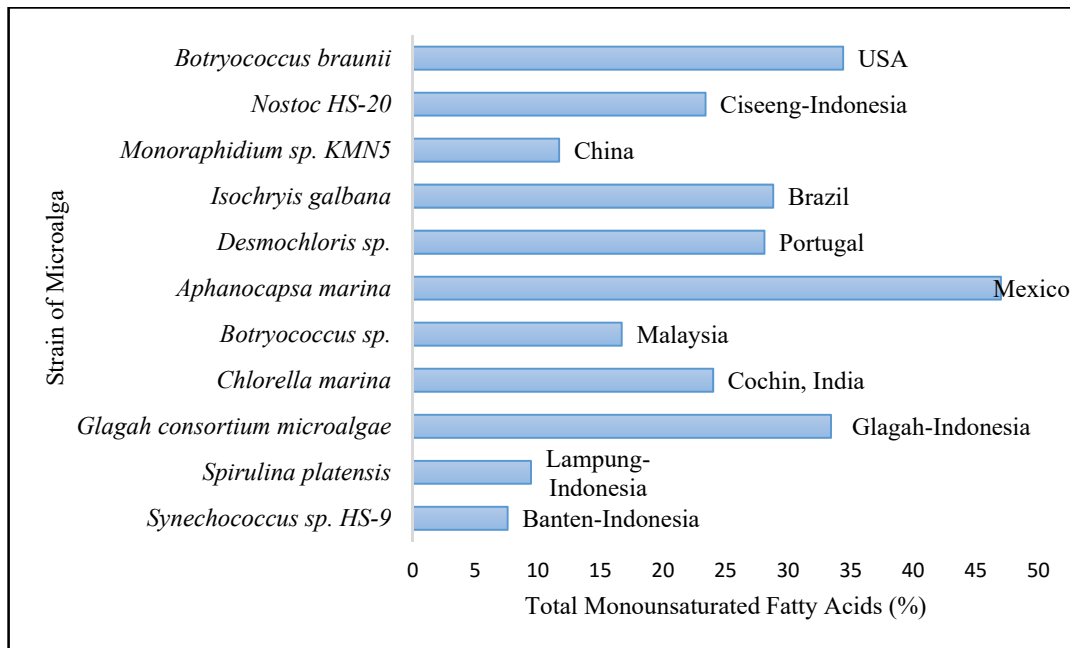


FIGURE. 2b. Total monounsaturated fatty acid (MUFA)

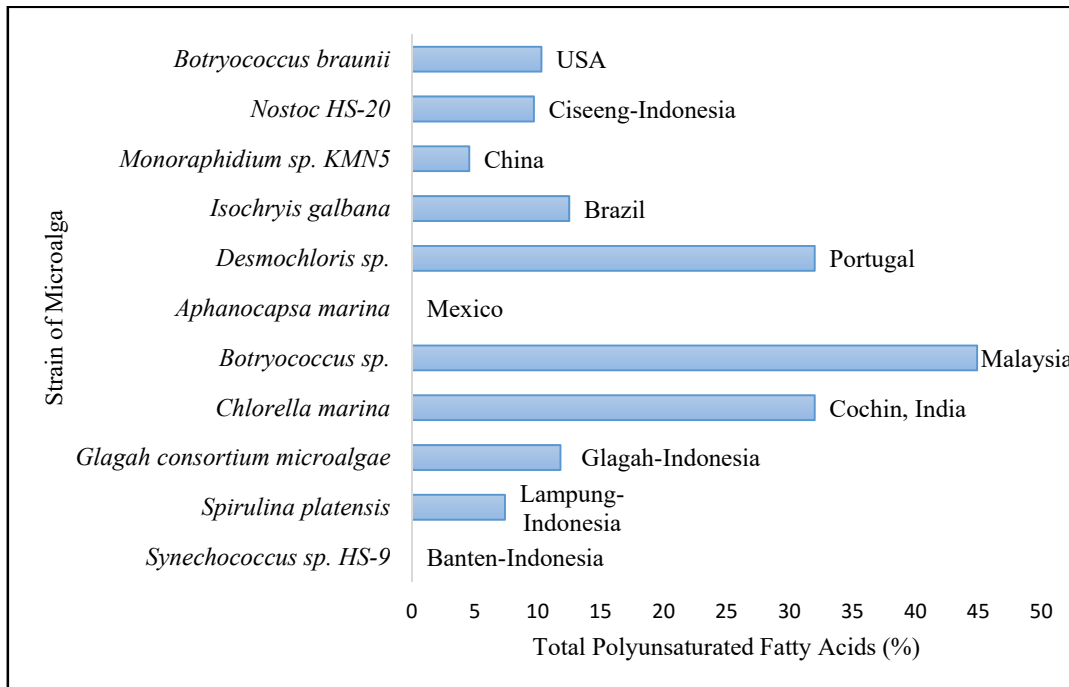


FIGURE. 2c. Total polyunsaturated fatty acid (PUFA)

STATUS OF BIODIESEL PRODUCTION FROM MICROALGAE IN INDONESIA

The Development of Microalgae Research

The resources and reserves of renewable energy in Indonesia are very large, but the development is not yet optimal. At present, the use of renewable energy is dominated by hydropower, in the form of hydroelectric power plants, and followed by biomass, geothermal and biodiesel. One of the most important things is increased use of biodiesel will reduce imports of diesel oil and saving the country's foreign exchange [24]. Indonesia has drafted the development of biofuels by setting a national energy supply of 5 % by 2025. This draft is in line with the International Energy Agency's estimates that biofuel will contribute 6 % of the world's total fuel use by 2030 [25]. One of the efforts of several countries to increase energy independence and avoid their national energy crisis is to prepare a potential alternative biofuel which is derived from microalgae biomass.

The efforts and development in the utilization of microalgae for biodiesel have been done by Indonesian researchers and scientists. The list of institutions who are working on various aspects of microalgae as though microalgae collected from natural vegetation which is used for biodiesel production in Indonesia is given in table 2.

TABLE 2. Status of research and development work on microalgae in Indonesia

No	Institution	Work on microalgae specific species	Research and development area	Reference
1	Department of Biology, Universitas Indonesia	<i>Synechococcus sp. HS-9</i> , <i>Nostoc HS-20</i>	Characterization of Indigenous Cyanobacterial strain	[13]
2	Department of Chemical Engineering, Universitas Indonesia	<i>Nannochloropsis oculata</i> , <i>Chlorella vulgaris</i>	Biodiesel synthesis	[26]
3	Gajah Mada University	<i>Botryococcus baunii</i> , <i>Nannochloropsis sp.</i> , <i>Arthrospira platensis</i> , <i>mixed culture microalgae</i>	Oil algae extraction	[27]

continued				
No	Institution	Work on microalgae specific species	Research and development area	Reference
4	Institut Teknologi Sepuluh November (ITS)	<i>Chlorella sp.</i>	Optimization of airlift photo bioreactor	[28]
5	Center for Research in Marine and Fisheries Product and Biotechnology Processing, Ministry of Marine Affairs and Fisheries	<i>Botryococcus baunii</i>	Biodiesel production	[29]
6	Department of Marine Science and Technology, Bogor Agricultural University	<i>Chlorella vulgaris</i>	Optimization of oil extraction	[30]
7	Environmental Engineering, Diponegoro University	<i>Spirulina sp.</i>	Production liquid fuel	[31]
8	Center for Energy Technology, Agency for the Assessment and Application of Technology (BPPT)	<i>Scenedesmus sp.</i>	Oil extraction	[32]
9	Research Center of Chemistry, Indonesia Institute of Sciences (LIPI)	<i>Wet BLT0404 microalgae</i>	Lipid extraction	[33]
10	Biochemistry Division, Faculty of Mathematics and Natural Sciences, Institut Teknologi Bandung	<i>Thalassiosira sp.</i>	Oil productivity of the tropical marine diatom	[34]

The Potential and Challenges of Microalgae Research

The enormous potential for energy resources in Indonesia such as microalgae which can be converted into biodiesel needs to be utilized efficiently [35]. The diversity of microalgae in the world is estimated to be in the range of millions of species, most of which have not been recognized and cannot be cultivated. It is estimated that 200.000-800.000 species live in nature, 35.000 species can be identified and 15.000 chemical components of biomass composition are known [36]. The potential of microalgae in Indonesia covers an area of 27.700 ha with a potential production of 462.400 tons/year [35]. In addition to biodiesel, the applications that can be utilized from microalgae are bioethanol, methane, food ingredients, pharmacy and carbon capture.

However, research on microalgae in Indonesia has not been optimal and is still laboratory scale. The problem are that there are few researchers who isolate microalgae from Indonesia, each strain of microalgae has different characteristics, so the cultivation process must be adapted to the characteristics of microalgae, and the operational costs for biodiesel production are very expensive. Therefore, further researches are expected to focus on developing culture with open or closed pond methods for microalgae cultivation, increased the economics of microalgae biodiesel by using biorefinary based production strategies, improved microalgae capabilities through genetic engineering, which can lead to major improvements and increase commercial feasibility of microalgae as an optimal biodiesel source. As mentioned, the use of microalgae as renewable energy source in Indonesia is very promising, but the biggest challenge faced is how to use microalgae biomass potential efficiently and develop appropriate technology.

CONCLUSION

The fatty acid composition of *Synechococcus sp.* HS-9, *Spirulina platensis*, *Glagah consortium microalgae*, and *Nostoc HS-20* from Indonesia could be a promising feedstock for biofuel production. The quantity of fatty acid microalgae from Indonesia higher than the other countries. However, the use of microalgae as renewable energy source in Indonesia is very promising, but the biggest challenge faced is how to use microalgae biomass potential efficiently and develop appropriate technology.

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