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Fatty Acid Content of Indonesian Aquatic Microalgae

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High utilization of fossil fuel increases the level of carbon dioxide in the atmosphere and results in global warming phenomenon. These things establish the world's thought to look for the other alternative energy that can reduce the use of fossil fuel even to be replaced by the substitute. Recently, Indonesia has been doing the research of microalgae as a feedstock of an alternative biofuel. Fatty acid content that microalgae have is also high to produce biofuel. The steps used in this research is a 7 days cultivation, harvesting, extraction using hexane, and fatty acid identification using Gas Chromatography of microalgae species. Fatty acid component in some species such as *Chlorella* sp., *Scenedesmus* sp., *Nannochloropsis* sp., and *Isochrysis* sp. is between 0.21-29.5%; 0.11-25.16%; 0.30-42.32%; 2.06-37.63%, respectively, based on dry weight calculation. The high content of fatty acid in some species of microalgae showed the potential to be the feedstock of producing biofuel in overcoming the limited utilization from petroleum (fossil fuel) presently.

Key words: fatty acid, marine, microalgae

INTRODUCTION

Microalgae are one of marine microorganisms that have high potency especially in developing alternative energy, as a substitute to fossil fuel and natural (Chisti 2007). According to Shay (1993), microalgae are organisms that can produce the largest biodiesel per hectare, up to 100 times higher than soybean oil. Biodiesel also serves as a complementary for the diesel fuel that is currently used. As a comparison, microalgae produce 7 to 30 times more oil compared to oil palm. Various energy products obtained from processing microalgae are: methane (Spolaore *et al*. 2006), biodiesel (Gavrilescu & Chisti 2005) and biohydrogen (Kapdan & Kargi 2006).

Previous studies on fatty acid substances in microalgae have been conducted by Yasar and Sevket (2006) on fatty acid content of *Spirulina platensis*, Chiu *et al.* (2009) on fatty content in *Nannochloropsis oculata*, and Qiang Hu *et al.* (2008) on triacylglycerol content in microalgae. These substances are important components in microalgae utilization as energy materials since the substances will be processed into fuel material. However, not all microalgae species contain potential fatty acid substances. This is especially, related to growth characteristics and ability to produce the substances. Some species, such as: *Botryococcus* sp., *Nannochloropsis* sp., *Isochrysis* sp., dan *Schizocytrium* sp. have been identified containing high fatty acids (Spolaore *et al.* 2006). Those species, however, can not easily be found in Indonesian waters.

The purpose of this study is to identify the hydrocarbon and fatty acids in some fresh water species as well as marine species. Furthermore, we expect to get the depiction of microalgae productivity in Indonesian waters as material sources to produce biofuel.

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MATERIALS AND METHODS

Microalgae Cultivation. Eight microalgae species from the collection of Surfactant and Bioenergy Research Center – IPB were cultured in a 1 litre Erlenmeyer glass using liquid medium with strain: medium ratio of 1: 9. We used nutrients from pro analysis chemical substances (Guillard and Conway media) depending the species. Cultivation was conducted in laboratory room temperature (23 ± 1 °C) with light radiation of 2,000 lux for 7 days to obtain maximum microalgae growth.

Microalgae Harvest. Microalgae were harvested when they have reached the stationery phase which is at day 7 using flocculation method by adding 120 ppm solid NaOH concentration to 1 litre cultivation of microalgae and followed by a filtration using a satin fabric. This harvesting process took 2-3 days to complete. Microalgae were dried by freeze dryer for about 2 days. The dry microalgae can be preserved for days until it can be processed further. Dry microalgae were weighted to obtain biomass weight value and ratio between dry weight and wet weight from the cultivation.

Fatty Acid Extraction. Extraction processes refer to the method developed by Indonesia National Standard Body/Standar Nasional Indonesia (1992). 20 grams of microalgae powder extracted using 150 ml hexane solvent put into a soxhlet device for 7 hours. The extracts were then purified by distillation to eliminate the solvent. For the preparation of fatty acid identification, 25 mg of the extracts was put in a tube and then added with 0.5 ml of

1 mg/ml standard internal solution (C17:0). Into the solution was added 1.5 ml NaOH 0.5N then the tube was filled with nitrogen gas and closed tightly, agitated and boiled in a steam-bath for 5 minutes at 100 °C, and then the tube was cooled. 2 ml methanolic BF3 (14% w/v) was added and refilled with nitrogen gas, covered tightly and heated at a temperature 100 °C for 30 minutes. Then it was cooled to the temperature 30-40 °C and 1 ml hexane was added for the preparation, then vortexed until homogen. Formed hexane layer was transferred to a vial and the water methanol phase was extracted again with 1 ml of hexane. The extract was filtered with anhydrous Na_2SO_4 and then concentrated with nitrogen gas until reaching 1 ml. The extract was ready to be analysed by using GC (Gas Chromatography).

Fatty Acid Analysis. The extracts were analyzed by using GC and GC-MS. GC analysis used Hewlett-Packard 6890 chromatography (split injector; 250 °C and flame ionization detector at 300 °C), fused silica column (Omegawax 250; 30 x 0.25 mm internal diameter dan 0.25 film thickness) and He gas as carrier. Program of oven temperature is 70-280 °C with the acceleration of 5 °C/ minute, and temperature was maintained 280 °C for 10 minutes. MS condition was full scan (m/z 50-600), cycle time 0.65 second and electron ionization at 70 eV. Injections were conducted 3 times. The results then were averaged to obtain its mean value. Methyl octanoat was used as standard solvent.

Quantification of Fatty Acids Concentration. The below equation used by U.S Environmental Protecting Agency (U.S EPA) was used in determining the hydrocarbon and fatty acids concentration (EPA 1996). The percentage (% dry weight) of fatty acid and hydrocarbon will be calculated using this equation. The equation is:

$$\frac{\text{mg of internal std}}{\text{gram oil}} \times Rf \times \frac{\text{fatty acid area}}{\text{internal std Area}}$$

RESULTS

Biomass Weight. Biomass weight of the microalgae species differed from each other. *Chlorella* sp. had the highest weight of 508 g DW (dry weight), while *Scenedesmus* sp. had the weight of 40 g DW (Table 1). As can be seen from the Table 1, the weight some microalgae species could not be measured since they contained too much salt in their cells. Water content of only some species can be measured, because the dry biomass weight is too light.

Table 1. Biomass weight (g DW) and water content (% biomass) of 10 microalgae species

Spesies	Biomass weight (g)	water content (% biomass)
Scenedesmus sp.	40	4.31
Chlorella sp	508	6.53
Nannochloropsis sp.	25	11.02
Isochrysis sp.	13	16.76
Dunaliella sp.	5	-
Spirulina sp.	-	-
Nitzschia sp.	-	-
Tetracelmis sp.	1.4	-
Chaetoceros sp.	3	-
Chaetoceros chalcitris	-	-

Chemical Substance Composition. Each species had different fatty acid content value. *Scenedesmus* sp. had fatty acid content of 0.07-35.52% dry weight with vinyl laureate as the highest concentration. While in *Chlorella* sp., the dominant fatty acid substance was valerate acid (with 10.06% dry weight).

Nannochloropsis sp., had methyl palmitate acid as the dominant fatty acid with concentration between 0.78-3.26% dry weight. No fatty acid was detected in *Dunaliella* sp.

Methyl oleate was the dominant fatty acid substance in *Isochrysis* sp., with concentration between 0.33-1.25% dry weight. And in *Nitszchia* sp., methyl palmitate was the dominant fatty acid substance with concentration between 0.05-11.52% dry weight.

Tetraselmis sp., had vinyl laureate as the dominant fatty acid substance with fatty acid concentration between 0.12-46.7% dry weight. In *Spirulina* sp., methyl oleate was the dominant fatty acid substance with concentration between 0.07-22.58% dry weight. The complete data of fatty acid concentration is shown in Table 2 and Figure 1.

DISCUSSION

Each species had different chemical compounds. From the analyses, it can be stated that *Tetraselmis* sp. contains the highest averaged concentration of fatty acid. while

Table 2. Fatty acid concentration (% dry weight) in 7 microalgae species

Contents	*Scenedesmus* sp.	*Chlorella* sp.	*Nannochloropsis* sp.	*Isochrysis* sp.	*Nitzschia* sp.	*Tetraselmis* sp.	*Spirulina* sp.
Capriat acid	0.07	-	0.30	-	-	-	0.07
Laurate acid	0.22	0.02	0.99	-	2.04	0.18	3.08
Myristate acid	0.34	-	7.06	0.33	1.30	0.12	2.00
Stearat acid	13.85	29.50	-	20.21	2.29	0.21	3.50
Palmitate acid	20.29	8.09	23.07	0.93	11.52	1.05	17.28
Oleate acid	-	2.41	12.25	37.63	14.80	1.40	22.58
Valerate acid	-	10.06	-	-	-	-	-
Margarita acid	-	-	-	0.77	0.05	-	-
Palmitoleat acid	9.78	2.15	42.32	34.25	0.07	-	0.24
Palmitolineat acid	-	-	-	-	0.16	-	-
Linoleate acid	25.16	45.07	2.47	2.06	6.37	0.57	9.93
Linolenate acid	16.16	11.49	-	-	-	-	-
Gliserol trilaurate	3.73	-	-	-	46.50	-	-
Vinyl laurate	35.52	-	-	-	46.70	-	-

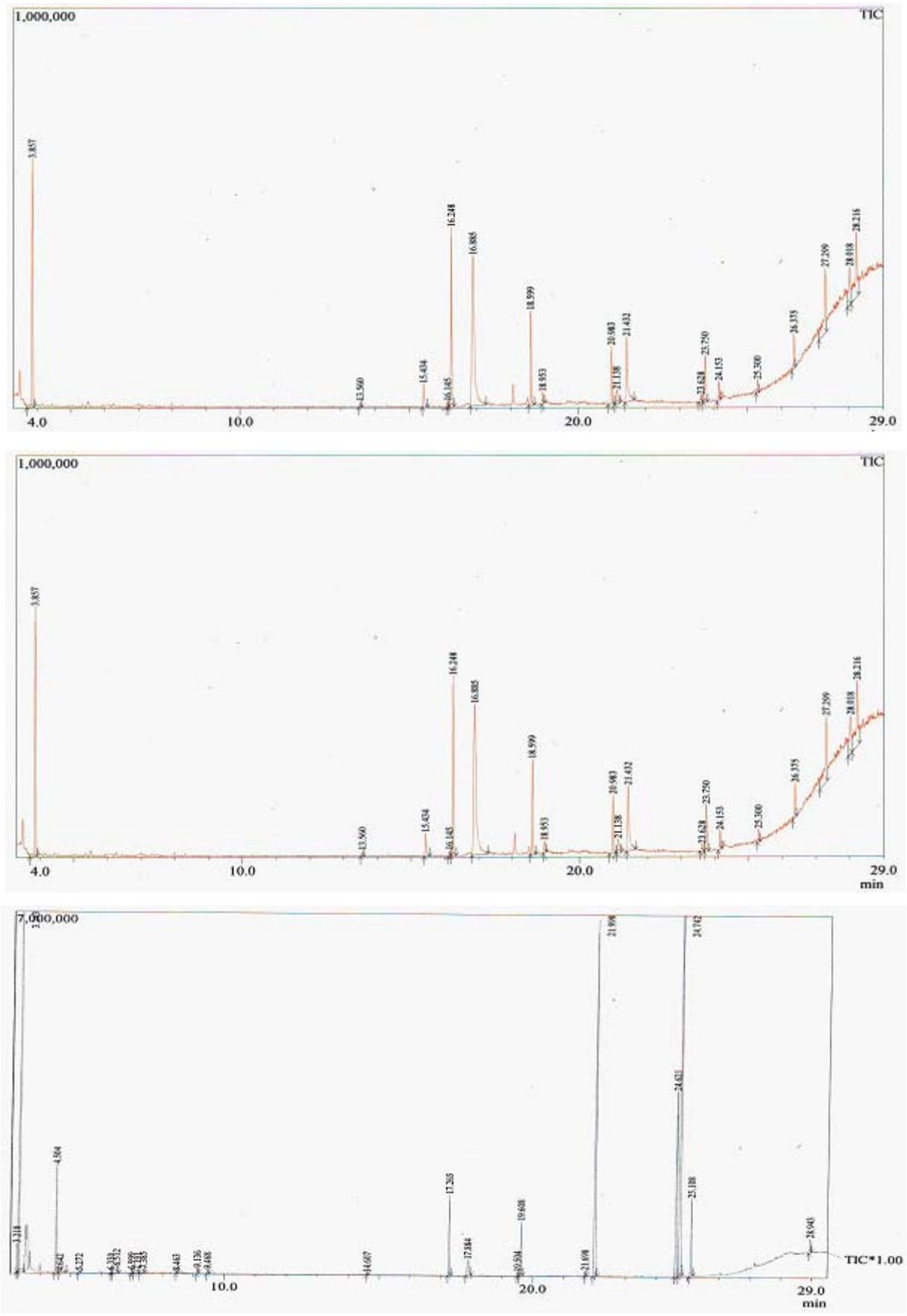

Figure 1. Example of chromatogram for 3 species (*Nannochloropsis*, *Scenedesmus*, *Spirulina*).

the lowest concentration is in *Dunaliella* sp. (undetected). The most common fatty acid type found was SFA (*Saturated Fatty Acid*) type, fatty acid that is commonly found in food. In *Scenedesmus* sp., the total of SFA content was 53.73% dry weight. whereas Pratoomyot *et al.* (2005) only found 22.22% dry weight at stationary growth stage, the total fatty acid of *Scenedesmus* sp. is much higher compared to the previous research.

Total fatty acid obtained in *Chlorella* sp. is 18.15% dry weight. According to Pratoomyot *et al.* (2005), total fatty acid content as many as 21.35% dry weight, which is higher compared to the value obtained in this research. In *Tetraselmis* sp., total saturated fatty acid content is 94.76% dry weight. Research conducted by Pratoomyot *et al.* (2005) gives total fatty acid value of 18.97% dry weight, thus this research resulted in much higher value of fatty acid content of *Tetraselmis* sp. *Tetraselmis* sp. also gives unsaturated fatty acid of MUFA (Mono Unsaturated Fatty Acids): linoleate dan oleate acid substance with total concentration of 1.97% dry weight. Pratoomyot *et al.* (2005) research's resulted 56.03% dry weight, which is much higher MUFA content, compared to this research (1.97% dry weight).

Saturated fatty acid detected in *Nitzschia* sp., is 17.36% dry weight. If compared to research conducted by Pratoomyot *et al.* (2005), this value is lower. Unsaturated fatty acid is also found in *Nitzschia* sp. with total content of 21.24% dry weight. Total of unsaturated fatty acid in *Nitzschia* sp. from research by Pratoomyot *et al.* (2005), is 65.53% dry weight, much higher that the value obtained form this research.

Total saturated fatty acis found in *Spirulina* sp., is 25.93% dry weight. If compared to 42.3-47.6% dry weight obtained by research by Muhling *et al.* (2004), then the obtained value is lower. Another research, conducted by Yasar and Sevket (2006) showed the saturated fatty acid content is 70.3% dry weight. This research gives MUFA composition for *Spirulina* sp. of 22.82% dry weight. If it is compared with the previous research by Yasar and Sevket (2006) that only resulted in 1.9% dry weight, this research has a higher value. For PUFA, this research gives 9.93% dry weight. This value is lower compares to the previous research by Yasar dan Sevket (2006) that resulted in 18.6% dry weight at 26 ^{o}C. The total fatty acid content in *Spirulina* sp. Is 58.68% dry weight.

Total fatty acid content in *Nannochloropsis* sp. and previous research show fatty acid content of *Nannochloropsis* sp. is between 30.8-50.4% dry weight (Chiu *et al.* 2009). This value is higher compared to the value obtained from this research. Total fatty acid content in *Isochrysis* sp. is 2.03% dry weight. If it is compared to the result obtained by Natrah *et al.* (2007), then this value is lower, where the value obtained by Natrah *et al.* (2007) is 14.5% dry weight. This also implies when 2.03% dry weight obtained from this research compared to George *et al.* (2008) research which gave the value of 28.9% dry weight. MUFA concentration in *Isochrysis* sp., is 1.25% dry weight (Oleate acid). Compared to research by George *et al.* (2008), that resulted 26.9% dry weight (total from 3 MUFA substances), 1.25% dry weight is lower.

According to the results, almost all the species from the recent research have different results from the previous references. This could be caused by place differences and also the medium that used for microalgae cultivation. Almost all of the species from this research was collected from Indonesian waters and the nutrients are different from the other aquatics area. Microalgae produce fats when they are stressed. Different from human, microalgae can accumulate fat when they feel starved and when they have used all the nutrient they had also when there are nutrient limitations in the environment. This condition can produce fats more for the microalgae cell and makes the fat concentration higher than before. Almost all of the results from this research has lower fatty acids concentration than the previous researches, which could means the nutrient of microalgae has no nutrient limitations and never been placed in an underpressure condition.

The potential of microalgae species in this research has been proved by the fatty acid concentration in some species. Some of the species have high concentration of fatty acid, the highest is recorded in *Nitzschia* sp. and the second highest is *Scenedesmus* sp. from this result, this two microalgae species has a big potency in producing fatty acids more than the other species and can be the raw material for producing biofuel from microalgae as the alternative source. One of the biofuel type is biodiesel and biodiesel is made from fatty acids of the raw material. The more of fatty acid in one species of microalgae, the bigger potency of the species to produce biodiesel. Compared to Teresa (2010), the most potential microalgae species that can be the source for biofuel is *Chaetoceros muelleri*, *Nitzschia* sp. is from the same class with *Chaetoceros muelleri* which is Bacillariophyceae (Diatom). This similarity can lead to the conclusion that Bacillariophyceae class of microalgae has a big potency to produce biofuel more than the other microalgae classes.

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