Lipid and fatty acid composition microalgae *Chlorella vulgaris* using photobioreactor and open pond

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**Abstract.** Microalgae contain lipids and fatty acids that can be the raw materials of biofuel. Previous studies have been known of using cultivation systems to obtain biomass of *C. vulgaris* which can be extracted to obtain lipid and fatty acid content. The observational step was observed ten days in photobioreactor and open pond for harvesting biomass using NaOH, lipid extraction using hexane and methanol, and fatty acid analysis using Gas Chromatography. Lipid content of microalgae biomass in photobioreactor and open pond was 2.26 ± 0.51% and 3.18 ± 0.80%, respectively. Fatty acid content ranged between 0.7-22.8% and 0.9-22.6% and the dominant fatty acids in both cultivating system was palmitic acid.

1. **Introduction**

Microalgae are microscopic organisms that live in fresh and marine waters and produce their own food through photosynthesis [1]. Microalgae contain lipids and fatty acids that can be the basic ingredients of biofuel [2]. Another advantage of microalgae does not compete with other crops in land use, otherwise it produces oil in larger quantities when compared to land plants in the same area [1]. The genus *Chlorella* is one of the most abundant types of microalgae in aquatic habitat, with green features, spherical structure, can grow well in the salinity range 0-35 psu, and the optimum temperature is 25-30°C [1].

Various advantages of microalgae make the organism potentially as industrial raw material, so much conducted the development of cultivation system and lipid extraction. The microalgae cultivation system influences the cultivation outcome of biomass, further extracting lipid and fatty acids [1]. A good cultivation system is needed to support the growth of microalgae so the optimum biomass can be known. In general, microalgae can be cultivated using photobioreactor and open pond.

Photobioreactor is a closed harvesting system that can be conducted indoor or outdoor. For indoor culture, fluorescent lamps is used as a light source, for photobioreactor that using sunlight as light source should be conducted in outdoor[1]. Photobioreactor is made using glass to facilitate light penetration. The application of photobioreactor has increased and developed for application in the pharmaceuticals,
cosmetics, aquaculture, and agriculture. Open ponds used to cultivate microalgae can be artificial ponds or natural ponds such as lakes. Decreased media due to evaporation, atmospheric CO₂ diffusion, and high risk of exposure to contaminants are a major problem in microalgae cultivation using open pond [3].

Lipid and fatty acid from biomass microalgae have similar chemical content to vegetable oil and considered as potential biofuel feedstock [4]. Microalgae lipid content has form of glycerol and fatty acids. Under certain conditions, microalgae are known have 85% of the dry weight lipids, this amount exceeds the amount of lipids if compared with the other land plant [1]. The purpose of this study is to analyze the biomass, lipid, and fatty acid content of microalgae C. vulgaris using two different cultivation system, the photobioreactor and open pond.

2. Materials and Methods
The study was conducted from August 2016 to January 2017. Microalgae culture was conducted in the laboratory of the Marine Bioprospecting and Biomaterial Department (MBB) of Marine Science and Technology and the laboratory of Surfactant and Bioenergy Research Center (SBRC). Microalgae seeds C. vulgaris was obtained from Surfactant and Bioenergy Research Center.

2.1. Microalgae cultivation procedure
The cultivation on the laboratory scale was conducted to multiply the microalgae seeds to reach the required amount for the next stage. Lab scale cultivation was carried out to the size of 1-5 liters per container. Walne fertilizer was given and microalgae placed under a TL lamp (1000 lux) for cultivation at this stage. Semi-mass cultivation is carried out from volume of 5 liters to 50 liters. The fertilizers used at this stage were replaced by the technical fertilizers referring to [1]. At the stage of mass scale cultivation, the process is conducted using photobioreactor and open pond. The light obtained comes from sunlight because mass cultivation was conducted outdoors. Mass cultivation was performed on 100 liters volumes for 10 days with the same volume in photobioreactors and open pond. Cultivation was conducted with twice repetitions based on time. The fertilizer used was TSP (CaH2PO4) 30 ppm, ZA (ammonium sulphate) 60 ppm and 60 ppm Urea which refers to [5]. While the comparison between microalgae and medium was 1: 10 [5].

2.2. Calculation of biomass weight
Biomass weight calculations are performed daily using gravimetric method. Whatman filter paper of 90mm diameter was inserted into an oven with 60°C temperature for 3 hours to remove the moisture present in the filter paper. Then cooled for 10 minutes and recorded the empty weight (b₀) on the digital scale. After filter paper used for microalgae biomass, put it back into the oven at the same temperature and time. After that it is cooled in the desiccator at the same time then weighed again and recorded the weight of its contents (b₁). The difference between (b₀) and (b₁) is the biomass of microalgae (g/L). Then put into equations referring to [6]:

\[
\text{Biomass (g/L) = } (b₁-b₀) \frac{1000}{\text{ml sample}} \tag{1}
\]

Information :

b₀ = filter paper weight before filtering
b₁ = filter paper weight after filtering
2.3. Analysis of lipid and fatty acid
The lipid extraction and calculation formula in this study refers to [7]. After dry biomass is obtained, the biomass was dissolved into hexane and metanol at a ratio of 1: 1, as much as 200 mL in erlenmeyer. The next stage was sonication 38 kHz for 20 minutes. The mixture was homogenized with a magnetic stirrer with a temperature 25°C for 24 hours. The extraction results were centrifuged at 4000 rpm for 10 min. The results obtained are supernatant and natant (pellet). Then the supernatant evaporated on a rotary evaporator with a temperature 50°C to obtain lipid from microalgae biomass. Then used extracted lipid microalgae for analyzed fatty acid composition based on methods issued by Environmental Protecting Agency (EPA 1996) with GC-MS [1]. Lipid from microalgae biomass calculated using formula [7]:

\[ L = \frac{M_l}{M_s} \times 100 \]  

(2)

Information :
- \( L \) = lipid content (%)
- \( M_l \) = lipid mass (g)
- \( M_s \) = sample mass (g)

3. Results and Discussions
3.1. Microalgae Biomass C. vulgaris
The current photobioreactor biomass (h0) is 0.43 ± 0.29 g/L. The biomass at open pond at (h0) is 0.41 ± 0.15 g/L (Figure 1). There is a fluctuation of microalgae biomass that is cultivated in both systems according to their life phase.

Photobioreactor biomass at (h0) or lag phase of 0.43 ± 0.29 g/L then increases to the highest value at (h4) or stationary phase is 1.27 ± 0.44 g / L. The current photobioreactor biomass (h5) weighing 0.93 ± 0.58 g / L then decreases to the end of the observation or when the microalgae reaches the death phase at (h10) weighing 0.34 ± 0.33 g / L. The open pond biomass at (h0) or lag phase is 0.41 ± 0.15 g/ L then increases to the highest value at (h5) or when the stationary phase is 0.94 ± 0.11 g / L. Biomass continues to decline to the end of observation when (h10) or when it reaches the death phase to be 0.49 ± 0.13 g / L.

There is an increase and decrease in biomass from the beginning of observation. Increase biomass (h0) to (h4) on photobioreactor, in open pond there is an increase in biomass from (h0) to (h5). This increase is a result of the availability of nutrients in the media so the biomass is still increased, while the decline in the value of biomass due to nutrients in the media is reduced. This is in accordance with the statement of [8] and [9], that biomass is influenced by nutrients in the media. [10] stated a decline in growth and biomass due to the number of cells that have a lot of competition in obtaining nutrients.
3.2. Lipid Content
Photobioreactor had a lipid percentage of 2.26 ± 0.51%, while the open pond had a lipid percentage of 3.18 ± 0.80% (Figure 2). In addition, differences in lipid content caused by environmental stress. Open pond has a wider surface than photobioreactor, so open pond has greater risk of evaporation. The occurrence of evaporation due to rising temperatures in the open by the sunlight. Another research showed that there is an increase in lipid content of the microalgae when the temperature rised from 25°C-30°C, this is because the microalgae accumulated high lipid levels under stressful conditions and stored them in triaglyceride (TAG) form in the lipid body [1].

The biomass of the C. vulgaris microalgae obtained from both cultivation systems then extracted using hexane and methanol chemical solvents to obtain lipid from microalgae. The microalgae did not produce large amounts of lipids in the lag phase, because in the first 3-4 days the microalgae were still active dividing, leaving energy and carbon stored in protein [12]. The decrease in nutrients in stationary phase causes the cell division to decrease gradually and cell begin to store their products in the lipid form [13]. According to [1] the lipid content of microalgae is usually present in the form of glycerol and fatty acids.

The microalgae C. vulgaris biomass from both cultivation systems was extracted using chemical solvents of n-hexane and methanol to obtain lipids. The solvent interacts with the microalgae biomass by penetrated the cell membrane and entering the cytoplasm, and the lipid is extracted out of the microalgae cell. [14] stated that the use of n-hexane or non-polar solvents is not strong enough to break the protein bonds with lipids, so a mixture of methanol or polar solvent is used to form hydrogen bonds that can break the lipid bond with proteins.
3.3. Content of C. vulgaris fatty acids

The content of fatty acid microalgae is an important component for the formation of industrial raw materials because fatty acids become the compositions of the lipids, the highest fatty acid cultivation techniques are potentially used for larger cultivation scales [1].

The highest fatty acid analysis on photobioreactor is palmitic acid (C16: 0) 22.8% (Table 1). Stearic acid (C18: 0) 21.6%. Other fatty acids have a smaller percentage, such as linolenic acid (C18: 3) 14.9%, palmitoleic acid (C16: 1) 10.2%, oleic acid (C18: 1) 6.9%, linoleic acid (C18: 2) 6.9%, myristic acid (C14: 0) 6.3%, eicosapentaenoic acid (C20: 5) 6.2%, arachidonic acid (C20: 4) 2.0%, lauric acid (C12: 0) 0.7%. Open pond has a content of palmitic acid (C16: 0) 22.6%. Stearic acid (C18: 4) 21.4%. Other fatty acid components have a smaller percentage, such as linolenic acid (C18: 3) 14.3%, palmitoleic acid (C16: 1) 10.4%, oleic acid (C18: 1) 6.9%, linoleic acid (C18: 2) 6.6%, myristic acid (C14: 0) 6.1%, eicosapentaenoic acid (C20: 5) 6.0%, arachidonic acid (C20: 4) 2.3%, lauric acid (C12: 0) 0.9%.

The results of the study of C. vulgaris microalgae by [15] using wastewater had palmitoleic acid (C16: 1) of 30.54%, higher when compared with 10.2% photobioreactor and 10.4% open pond. Stearic acid (C18: 4) was 13.65% lower when compared with photobioreactor of 21.6% and open pond by 21.4%. There is no linolenic acid (C18: 3), when linolenic acid [18:3] at photobioreactor 14.9% and open pond 14.3%.

[16] used *Nannochloropsis gaditana* microalgae has a content of palmitic acid (C16: 0) of 53.4% higher than photobioreactor of 22.8% and open pond 22.6%. The content of linolenic acid (C18: 3) was 0.8% smaller than this study, with photobioreactor of 14.9% and open pond at 14.3%. The results of [16] did not find the content of stearic fatty acid (C18: 4), while in photobioreactor 21.6% and open pond 21.4%.

Previous research has shown that microalgae of different species has different fatty acid content, while microalgae with the same species with different cultivation conditions has different fatty acid content. [2] stated that differences in fatty acid content in microalgae due to differences used in initial conditions, techniques, and cultivation media.

The saturated fatty acids content *C. vulgaris* cultivated using photobioreactor and open pond is quite high when compared with unsaturated fatty acids, thus having the potential to be biodiesel. According to [2], high levels of unsaturated fatty acids are not suitable for bio-fuels because at the time of the production process still have to do the fatty acid oxidation balance. Production of biodiesel containing high unsaturated fatty acids is more susceptible to oxidation and will cause rancidity when compared to saturated fatty acids.

Fatty acid analysis of photobioreactor and open pond showed high content of palmitic acid (C16: 0), stearic acid (C18: 0), and linoleic acid (C18: 2). According to [17] fatty acid components that support for biodiesel are palmitic acid (C16: 0), stearic acid (C18: 0), and linoleic acid (C18: 2). [18] states that high palmitic acid and oleic acid can help the formation of biodiesel with better quality.
Table 1  *C. vulgaris* microalgae fatty acid composition

| Fatty acid (content)                | SFA/MUFA/PUFA | photobioreactor | open pond |
|-------------------------------------|---------------|-----------------|----------|
| Lauric acid (C 12:0)                | Saturated     | 0.7%            | 0.9%     |
| Myristic acid (C 14:0)              | Saturated     | 6.3%            | 6.1%     |
| Palmitic acid (C 16:0)              | Saturated     | 22.8%           | 22.6%    |
| Stearic acid (C 18:0)               | Saturated     | 21.6%           | 21.4%    |
| Palmitoleic acid(C 16:1)            | Unsaturated   | 10.2%           | 10.4%    |
| Oleic acid (C 18:1)                 | Unsaturated   | 6.9%            | 6.9%     |
| Linoleic acid (C 18:2)              | Unsaturated   | 6.9%            | 6.6%     |
| Linolenic acid (C 18:3)             | Unsaturated   | 14.9%           | 14.3%    |
| γ-linolenic acid (C 18:3n6)         | Unsaturated   | 1.6%            | 1.8%     |
| Arachidonic acid (C20 : 4)          | Unsaturated   | 2.0%            | 2.3%     |
| Eicosapentaenoic acid (C20 :5)      | Unsaturated   | 6.2%            | 6.0%     |
| **Total fatty acid**                |               | **100.1%**      | **99.3%** |

4. Conclusions
The highest biomass in photobioreactor at (h4) 1.27 ± 0.44 g/L while at open pond (h5) 0.94 ± 0.11 g/L. The biomass from both cultivation systems has the potential to produce lipids and fatty acid. The lipid content of open pond is higher when compared to photobioreactor. Photobioreactor had a lipid percentage of 2.26 ± 0.51%, while the open pond had a lipid percentage of 3.18 ± 0.80%. Photobioreactor had total percentage fatty acid 100.1% with the highest fatty acid analysis is palmitic acid (C16:0) of 22.8%, open pond had total percentage fatty acid 99.3% with the highest fatty acid analysis is palmitic acid (C16:0) of 22.6%.

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