

The Effect of Salinity on Growth, Dry Weight and Lipid Content of the Mixed Microalgae Culture Isolated from Glagah as Biodiesel Substrate

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Abstract: Microalgae use photosynthesis to convert solar energy into chemical energy, such as lipid and they can be a replacement for oil-based fuels. They are among the fastest growing plants in the world, and about 50% of their weight is oil. This lipid oil can be used to make biodiesel. Unfortunately, there are only some of potential strains isolated from Indonesia and most of the biodiesel productions are usually using a single strain. Then, although they are rich of oils, their biomass productivity is still low. Salinity treatment can be used to increase their biomass as well as their lipid content. Therefore, the research aim was to study the effect of salinity on the growth, dry weight and lipid content of mixed microalgae isolated from Glagah, Yogyakarta. The mixed microalgae were cultured in 3NBBM medium with different salinities or types of water (sea water, brackish water, and fresh water). The cultures were incubated at light intensity 3,000 lux under dark:light exposure of 12:12 h for 7 days. The number of cells was counted every 24 h with a Haemocytometer, and the biomass was calculated based on the dry weight. The lipid content was measured on days 0, 3, and 7 using NR (Nile Red) staining, and then the amount of lipid was analyzed using a fluorescence microscope and measured with CellProfiler 2.0 software. The highest dry weight and lipid content were found in seawater medium, they accounted for 3.42 mg/mL and 13.58% at day 7, respectively. Whereas, the highest number of cells was found in freshwater medium, this was 9.8×10^6 cells/mL.

Key words: Salinity, growth, dry weight, lipid content.

1. Introduction

The worldwide increase of human population and transportation has generated greater energy consumption of petroleum fossil fuels that lead energy crisis because of depleting fossil fuel reserves [1]. Therefore, for solving the issue above, a renewable energy as an alternative resource should be developed. Microalgae-based biodiesel production could be a potential source for the future renewable energy [2]. Microalgae as a potential candidate for biodiesel production has generated significant interest [3, 4], because the organism is the most efficient biological

producer of oil and biomass source due to use photosynthesis to convert solar energy and combine water with fixing CO₂ into chemical energy, such as lipid and it can be a replacement for oil-based fuel [5, 6].

Currently, biodiesel production were dominated by commercial single strains microalgae [3]. Most of the commercial single cell cultivations of microalgae have low biomass production and lipid content due to various problems such as culture condition and susceptibility to contaminant [7]. Those problems can be overcome through the search and selection of the local microalgae strains for biodiesel production which have highest growth rate, biomass productivity, and lipid content [8, 9]. Furthermore, the use of mixed

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culture of the selected local strains shows that cells grow faster and high biomass yield because the cells are capable for utilizing organic carbon sources optimally [10].

Even so, optimization technology is commonly done by regulating environmental conditions and culture medium [11]. By adjusting the salinity was reported able to increase biomass production, such as in *Scenedesmus almeriensis* culture [12] and *Scenedesmus* sp. culture [13]. Moradi and Ismail [14] also reported that *Botryococcus braunii* adapted to grow in low salinity was able to increase the biomass production, hydrocarbon, fat, carbohydrate, and carotenoids. Microalgae have a response against increase of salinity and osmotic stress on the environment by accumulating small molecules components for osmoregulation [6]. Hu [15] and Rao *et al.* [16] describe that the increasing of salinity leads to slight increase in the total lipid content of algae, but excessive salinity gives an negative effect on growth due to salt stress causes microalgae tend colonies-form in the growth phase, inhibit the photosynthesis and decrease the growth rate.

Therefore, it is important to study the effect of different salinities in culture medium of the mixed microalgae culture isolated from Glagah cell on growth, dry weight, and lipid content for biodiesel substrate.

2. Materials and Methods

2.1 Algal Cultures

The mixed microalgae culture isolate Glagah were obtained from Glagah beach in the coastal area of South Yogyakarta-Indonesia. Microalgae samples were isolated in Laboratory of Biotechnology, Faculty of Biology, UGM by using the microcapillary pipette method and serial dilution method [17]. The isolated microalgae was not identified yet, however, according to morphological characters, they consisted of three genus, there were *Chlorella*, *Scenedesmus* [18], and *Nannochloropsis* [19].

The strains were cultivated in modified sea water medium of f/2 for selection. Then, survived strains were cultivated in modified 3NBBM medium + vitamins medium [20] They were grown in 500 mL glass bottles and incubated at light intensity of 3,000 lux under dark:light exposure of 12:12 h for 7 days at 18-25 °C.

2.2 Cultivation Condition with Different Salinity

For all experiments, the microalgae were grown in modified 3NBBM medium + vitamin in 500 mL glass bottle under condition described above. The effect of salinity was investigated at 3NBBM + vitamins diluted in fresh water, sea water, and brackish water (mixture of fresh water:sea water (1:1)) with three replications. The cultures were inoculated into glass bottles of different salinity medium with ratio 50 mL stock culture and 200 mL the medium.

2.3 Determination of Cells Growth

To compare cell growth in different salinity, cells were counted using a light microscope and Haemocytometer Neubauer 1 mm every 24 h. Sample was shaken to homogenize, then 900 µL of sample put into microtube 2 mL mixed with 100 µL of alcohol 70% for fixing. Number of cells was calculated as follows:

$$\text{Cell number} = \text{cell count} \times 5 \times 10^4 \text{ cell/mL}$$

2.4 Determination of Algal Biomass

The biomass production was measured based on dry weight of the culture for seven days. Sample culture (2 mL) was transferred into microtube 2 mL. Sample was centrifuged at 3,300 rpm for 10 min. Supernatant was discarded, then washed using distillate water. Cell suspensions on micro tube bottom were dried in the incubator oven at 34 °C until having constant weight.

2.5 Determination of Lipid Content by Nile Red Staining

Lipid content was measured by using Nile Red

(9-(Diethylamino)-5H-benzophenoxazin-5-one) staining [21]. Nile red staining was conducted to detect intracellular lipid droplets [22]. The cultured cells (1 mL) were collected into micro tube and 0.01 mg/mL then was added with Nile Red for staining. Stained microalgae cells were observed by Fluorescent Microscope to get the lipid fluorescence. The lipid fluorescence from neutral lipid will be smeared yellow-orange [21, 23]. Then, lipid fluorescences were quantified using image analysis software CellProfiler 2.0 as an intracellular lipid droplet [24].

3. Results and Discussion

The comparison of, cell growth, dry weight, cell quota and lipid content in mixed cultures of microalgae isolated from Glagah, Yogyakarta using various salinities of medium in batch cultures were investigated. Those data are presented in Figs. 1a-1d.

As can be seen from Fig. 1a., the microalgae were growth better in fresh water medium as similar to their habitat. The highest number of cells was found in freshwater medium reached 9.8×10^6 cells/mL at day 7, followed by brackish water and sea water treatments. However, the highest total dry weight was found in sea water treatment accounted for 3.42 mg/mL, followed by brackish water and fresh water. Both sea water and brackish water treatments reached a pick at day 5, but fresh water treatment was at a pick at day 4. (Fig. 1b). It can be assumed that microalgae growth in fresh water medium were stimulated to proliferate their cells rather than construct their cell walls. The fresh water nutrient absorbed by microalgae cells did not used much to construct the cell wall, but to stimulate cell division. Then, their cells became smaller and their cell walls were relatively thinner and lighter. On the other hand, sea water nutrient provided more and suitable compounds for producing carbohydrate stored in the cell walls as cellulose and hemicellulose, so that the total dry weight was higher.

The trend of cell quota (dry weight per cells) of each treatment (Fig. 1c) was different from total dry weight (Fig. 1b). The highest cell quota was in the brackish water treatment at day 5 with reached 3.79 ng/cell, followed by sea water treatment and fresh water treatment, they accounted for 1.52 ng/cell and 0.17 ng/cell, respectively (Fig. 1c). Therefore, the total dry weight in sea water was the highest, but the dry weight per cells in brackish water was the highest. This happened because the cell division in brackish water was growth very slow (Fig. 1a).

Lipid contents of the microalgae in all treatments tend to increase during their growth. The highest lipid content of the microalgae was found in seawater treatment at day 7 accounted for 13.58%, followed by brackish water treatment (5.86%), and the lowest was found in fresh water treatment (0.88%) (Fig. 1d). Increasing of lipid content was caused by the accumulation of lipid under stress conditions. These results were similar to the research done by Takagi *et al.* [25] in *Dunaliella* cells. This was also confirmed by Hu [15] that the increase of salinity could accelerate the total lipid content in microalgae cells. Under high salinity condition, the microalgae were stimulated to enhance their lipid production as osmoprotectant which necessary to protect them from salt stress [26]. High salinity water might effect on intracellular osmolarity due to hypertonic medium condition over the microalgae cells. Then, it caused release of water inside of the microalgae cells into the environment. The mechanisms for encountering salinity stress were regulated by producing lipid in large quantities to prevent the escape of water from microalgae cells. So, the high amounts of lipid lead to enhancing the microalgae dry weight. Therefore, the high salinity treatment could be used as method to increase the biomass productivity and stimulate lipid production of microalgae for biodiesel substrates.

4. Conclusions

Different salinity have potential for increase the

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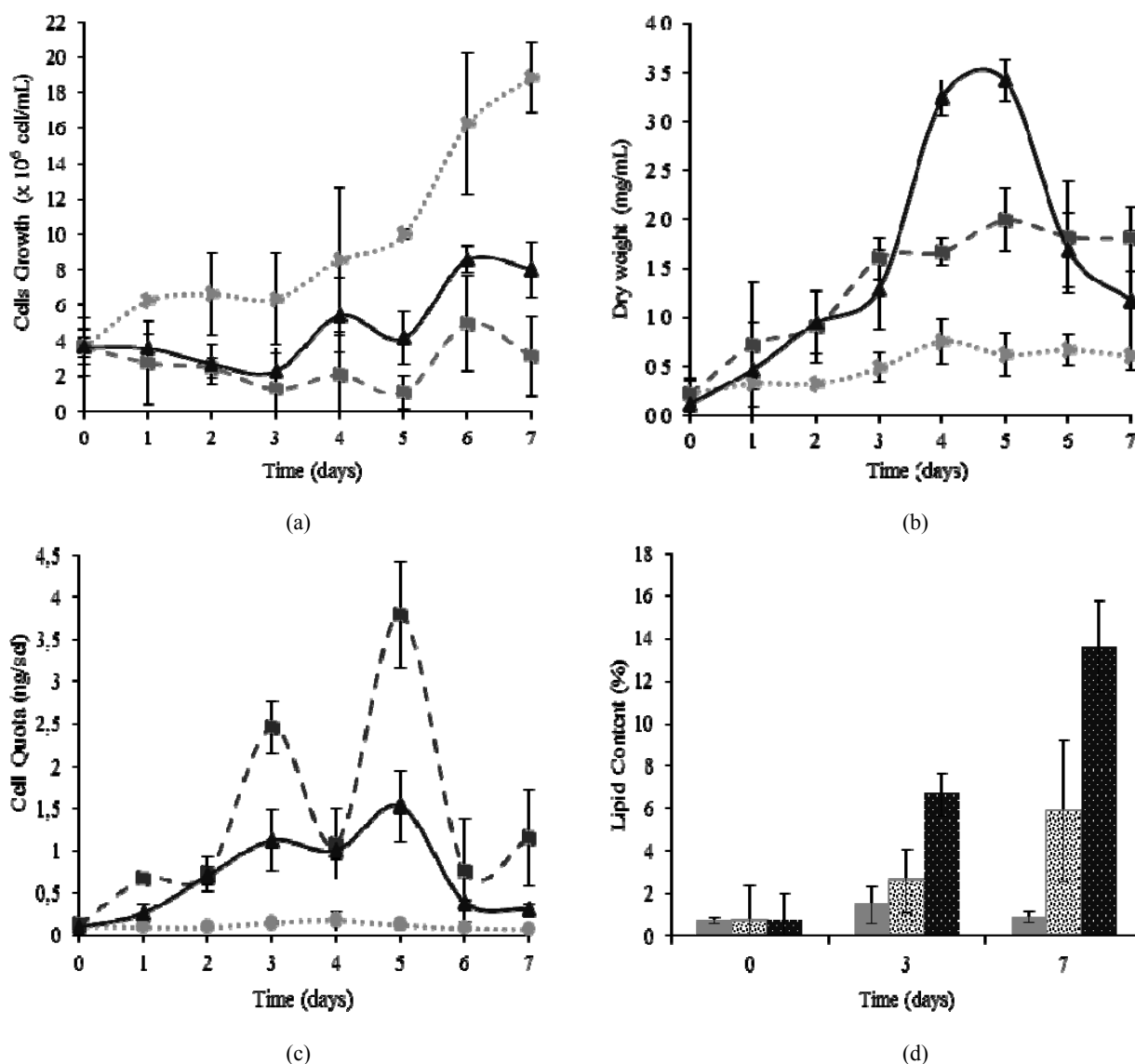


Fig. 1 Growth (A), dry weight (B), cell quota (C) and lipid content (D) of the mixed microalgae cultures isolated from Glagah were cultured in fresh water (●), brackish water (■), and sea water (▲).

number of cells, dry weight and lipid content on the mixed microalgae cultures isolates from Glagah. The highest dry weight and lipid content were found in seawater medium, they accounted for 3.42 mg/mL and 13.58% at day 7, respectively. Whereas, the highest number of cells was found in freshwater medium, this was 9.8×10^6 cells/mL.

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