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GRADUATE RESEARCH ARTICLE

Effect of Salinity Difference on Lipid Content from *Chaetoceros muelleri* on Continuous Reactors

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ABSTRACT

Chaetoceros muelleri is a microalgae class of *Bacillariophyta (diatom)* which is generally only used as feeds for fishes and shellfish larvae. Nevertheless, the biochemical content of this species is quite high and has the potential to be developed. This research aims to explain the effect of different salinity on the growth and lipid content of *Chaetoceros muelleri* cultured in a continuous photobioreactor. This research was carried out in August 2018 - February 2019. The research was conducted at the Laboratory of Marine Microbiology and the Laboratory of Bioprocess and Bioprospection of Natural Materials, Faculty of Fisheries and Marine Sciences, Padjadjaran University. The samples of *Chaetoceros muelleri* isolates were obtained from the Jepara Brackish Water Aquaculture Center. The methods used for the study was a 'Completely Randomized Design' (CRD) with four treatments. The salinity used is 15, 25, 35 and 45 ppt. The main parameters observed were growth and lipid content, while the supporting parameters were temperature, and pH. The results of this study showed that the highest lipid content was a salinity treatment of 35 ppt with a value of 25.37% of total dry weight obtained at the end of the culture. Based on growth, the highest density occurred in 25 ppt salinity with a maximum density of 3.80 ± 0.49 x 106 cells. ml-1 and maximum growth rate of 0.36 ± 0.008 div. day-1

Keywords: *Chaetoceros muelleri*, Lipid, *Photobioreactor*

1 Introduction

Microalgae are photosynthetic microorganisms that live in fresh or salty waters. Its presence in nature serves as the first source of nutrition in the food chain. Compared to other photosynthetic organisms, microalgae have a higher lipid content. The production of lipids produced by microalgae also 10 - 20 times greater per unit area compared to agricultural products [1].

Marine microalgae species, especially diatoms, are very promising in producing high lipids [2]. One of them is *Chaetoceros muelleri*. This species belongs to the genus *Chaetoceros* which is the largest type of phytoplankton diatom spread throughout the ocean [3]. This genus is cosmopolitan and has a fairly high salinity tolerance range of 15-45 ppt. Nowadays, *C. muelleri* is generally only used as feed for fish and shellfish larvae [4]. Nevertheless, the biochemical content of this species is quite high and has the potential to be developed.

The biochemical compounds contained in microalgae are largely determined by their environmental conditions [5]. Poor environmental conditions will trigger microalgae cells to adapt so that they will change the rate of biosynthesis and biochemistry. Some environmental factors that influence it include temperature, salinity, nutrients, light intensity and degree of safety [5].

Among the factors above, the use of salinity to increase lipid content has its advantages, namely, it is easy to apply on a large scale and low cost. High salt levels cause a series of bioenergy and biochemical changes in photosynthetic organisms. Among them, the most common thing is an increase in lipid biopolymers and changes in the permeability of plasma membranes [5].

Microalgae cultures on a laboratory or industrial scale generally use a bioreactor [6]. The type of reactor that is often used is Photobioreactor. There are two types of a photobioreactor, batch and continuous. The use of batch reactors in microalgae cultures can only maintain a culture for a few days due to nutrient limitations. Meanwhile, the continuous reactor can maintain a longer stationary phase because it can supply nutrients continuously. This can make the accumulation of lipids in microalgae cells can occur optimally. Thus, increasing lipid production from microalgae become more effective

2 Materials and Method

2.1 Continuous culture of *Chaetoceros muelleri*

Continuous culture of *C. muelleri* was carried out in a photobioreactor using 2 liters of *C.muelleri* culture which had been mixed with NPSi nutrient. The initial culture density in each salinity treatment (15, 25, 35 and 45 ppt) was 1 x 10^6 cells. ml^{-1}. The culture was included in each reactor box which had been given 6 L $minute^{-1}$ aeration. Lighting is set at 3000 lux and nutrient flow is set at 1 ml. day^{-1}. Observation of water quality parameters and growth is carried out every day for 30 days.

2.2 Continuous culture of *Chaetoceros muelleri*

The observed growth parameters were density, growth rate, and optical density. The density measurements were carried out using a Hemocytometer by taking 100 µL of culture using a micropipette. Cell counts are calculated using the following formula:

$$K = \frac{Average\ cell\ in\ one\ chamber}{Chamber\ volume} \times f$$

Where K is the value of density and F is the dilution factor. The growth rate is calculated using the following equation:

$$Growth\ rate\ (\mu)\ (day)^{-1} = \frac{\ln({}^{F_1}/_{F_0})}{t_1 - t_0}$$

Where F1 is the amount of biomass at the time (t1) and F0 is biomass at the time of initial observation (t_0). While the optical density was measured using a spectrophotometer at a wavelength of 750 nm.

2.3 Harvesting of Biomass

Harvesting of biomass is carried out in three phases namely exponential phase, stationary initial phase and end of culture. Harvesting of the culture is done by separating 180 ml of culture liquid with biomass. The separation process uses centrifugation at a speed of 2000 rpm for 10 minutes. Then proceed with a drying of biomass.

2.4 Lipid extraction

Lipid extraction from microalgae was carried out with the modified Blight-Dyer method. The extraction stage begins by weighing 100 mg of biomass powder and mixing it into 6 ml of 1: 1 chloroform-methanol solution. Then mix the mixture using vortex for 30 seconds. Re-added 2 ml of 1: 1 chloroform-methanol solution and 2 ml of distilled water. The mixture was centrifuged for 10 minutes at a speed of 2000 rpm. Centrifugation results will produce two layers, the top layer, and the bottom layer. The top layer then discarded and the bottom layer is transferred to the tube. The remaining pellets are extracted again using 4 ml of 1: 1 chloroform-methanol ratio and filtered using filter paper. The results of pellet extraction were put together in a previous tube and then evaporated at 61°C. The percentage of the lipid content was determined by the following formula:

$$\% \text{ Lipid} = \frac{\text{Total Lipid Weight}}{\text{Initial weight of biomass}} \text{ x } 100\%$$

3 Result and Discussion

3.1 Growth *Chaetoceros muelleri*

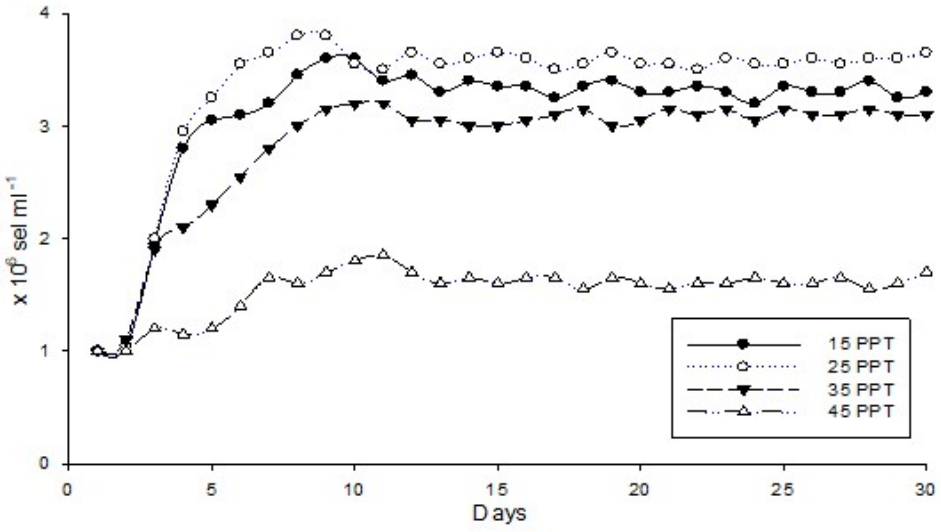

Figure 1: *C.muelleri's growth curve during continuous culture*

Based on the figure 1, it is known that the duration of the lag phase in all four treatments only occurs one day. This shows that isolates adapt faster. During continuous culture there is no phase of death, which is a special feature of continuous culture. The results of continuous culture (figure 1) show the highest density is in the salinity of 25 ppt with a maximum density reaching 3.8 x 10^6 cells. ml^{-1} and the lowest density at salinity 45 ppt with a maximum density of only 1.85 x 10^6 cells. ml^{-1}. This is similar with the research conducted by Barros et al. which showed that the optimal growth of *C.muelleri* in his research occurs in salinity 25 ppt [2].

The density curve obtained is regressed with the optical density measurement curve as validation. The regression results show that each of each treatment shows a value of R> 0.78 which means there is a strong relationship between density and OD.

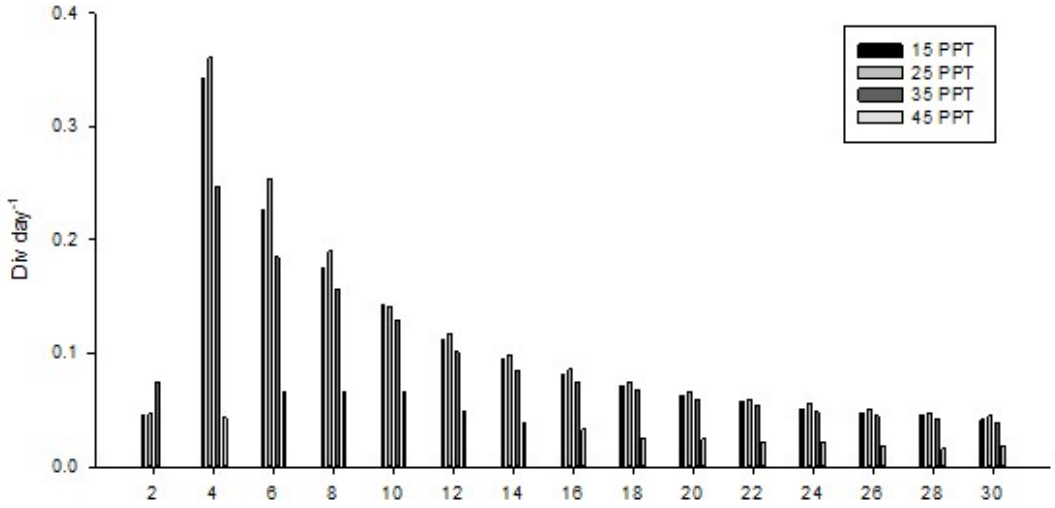

Figure 2: *C.muelleri's growth rate during continuous culture*

The maximum growth rate also shows the same thing, where the highest value is found in salinity treatment 25 ppt at 0.36 ± 0.008 div.days^{-1} and the lowest at 45 ppt treatment at 0.09 ± 0.073 div.days^{-1}. Based on the figure 2, the maximum growth rate of treatment 15, 25 and 35 ppt was on the 4th day while the 45 ppt treatment was achieved on the 8th day. The ANOVA test results for each salinity treatment on density and growth rate show that the value of $p < 0.05$, which means there is a real effect of salinity on density and growth rate

Salinity is one of the limiting factors in the growth of microalgae cells. Salinity can influence osmoregulation activity from cells [7]. When microalgae are at too high a salinity condition, the presence of nutrients for cell growth cannot be absorbed optimally because microalgae carry out the osmose process to maintain the pressure of their cell turgor and tend to store their energy [8]. This process will make cell division slower. This was also shown by Ishika et al. on some spesies diatom which showed that at high salinity concentrations there was a decrease in dry weight of biomass [8].

The reduction in growth in high salinity was caused by a decrease in the rate of photosynthesis in several species of marine microalgae [9]-[10]. This decrease in the rate of photosynthesis is closely related to the activity of PSII (Photosystem II). According to Pancha et al as long as cells adapt to high salinity PSII activity will decrease which can be caused due to damage to the PSII acceptor side and at the PSII reaction center [11].

3.2 Lipid Content *Chaetoceros muelleri*

Calculation of lipid content was also carried out in the three phases of *C.muelleri's* growth, namely the exponential phase (5th day), the stationary initial phase (10th day) and the stationary end phase (30th day). The results of the calculation of each phase were then statistically analyzed using ANOVA followed by a follow-up test at a significant level of $p = 0.05$. Statistical results of lipid content showed that the value of $p < 0.05$, which means there was a real effect of salinity on the lipid content in each treatment of each phase. The research results showed that there was an increase in lipid content in each treatment along with the length of the growth phase (figure 3). In the exponential phase, it was seen that the highest lipid content was found in the treatment of 15 ppt of 13.43 ± 0.81% and the smallest content was found in treatments 35 and 45 ppt where both had almost the same lipid content, 10.67 ± 1.21% and 10.47 ± 1.10%. In this phase, the lipid content is lower than in the other phases. During the exponential phase, microalgae tend

to use nutrients for the process of division and protein synthesis [12]. Proteins during this phase are needed to form cell structures.

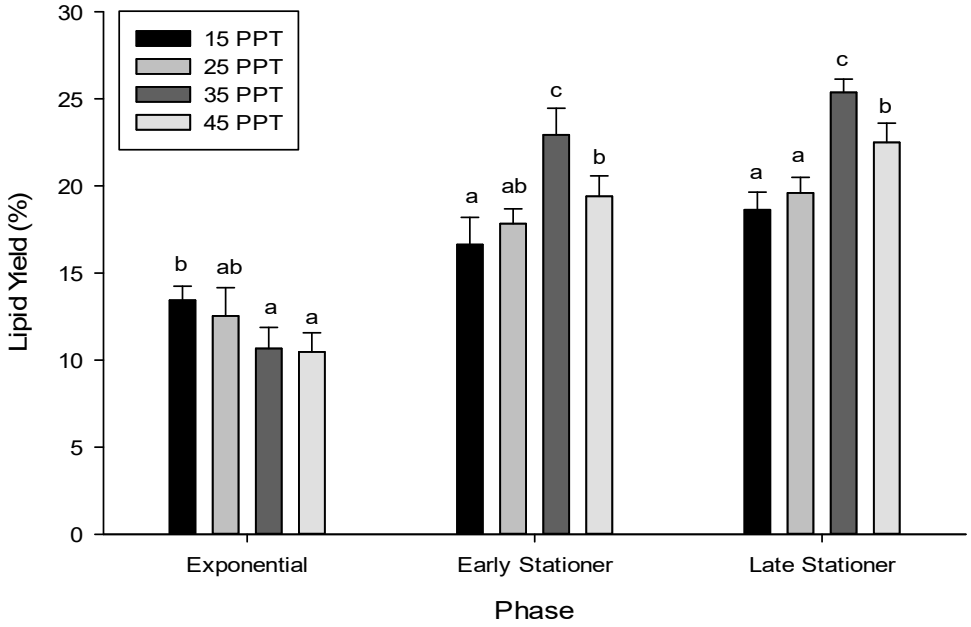

Figure 3: *Lipid Content Chaetoceros muelleri*

Based on figure 3, the initial stationary phase of four treatments shown an increase in lipid content of ± 3 - 12%. In this phase, the highest lipid content was found in the treatment of 35 ppt at 22.93 ± 1.53% while the lowest at treatment 15 ppt at 16.63 ± 1.56%. This phase is the phase where the value of cell density is at its peak. Ishika et. al explained that productivity biochemical compounds such as protein and lipids from several species microalga will increase linked increasing cell density until it reaches optimal density [8].

In the final stationary phase (figure 3), the four treatments showed a slight increase in lipid content from the previous phase. Increased lipid content ranges between ± 2-3%. The highest lipid content in this phase is still in the salinity treatment of 35 ppt of 25.37 ± 0.76% and the lowest is in the treatment of 15 ppt of 18.63 ± 1.01%. The lipid content in this phase is the highest compared to other phases. According to Barros et.al, the lipid content will increase dramatically in the stationary phase along with decreasing levels of nitrogen and silicates [2]. However, in continuous cultures, nutrient flow is always constant every day so that increased lipids during this phase are thought to be due to the cell's response to salinity. Salinity is one of the environmental factors that affect the growth of microalgae. Poor environmental conditions (outside of optimal conditions) will trigger microalgae cells to adapt so that they will change the rate of biosynthesis and biochemistry [13].

One of the compounds that can be affected is lipids. When cells adapt to salinity conditions, the cell will carry out an osmosis process. During this process, most microalgae will accumulate polyol compounds [13]. One of the polyol compounds commonly found in microalgae is glycerol. Glycerol is a compound needed to form neutral lipid such as triglycerides (TAG). Triglycerides are generally accumulated by microalgae as a backup energy source when the environment is outside optimal conditions [11]. Increased neutral lipid or TAG content along with increased salinity also occurs in several species such as Tetraselmis suecica [11] and Scenedesmus sp. [14].

3.3 Water quality parameter

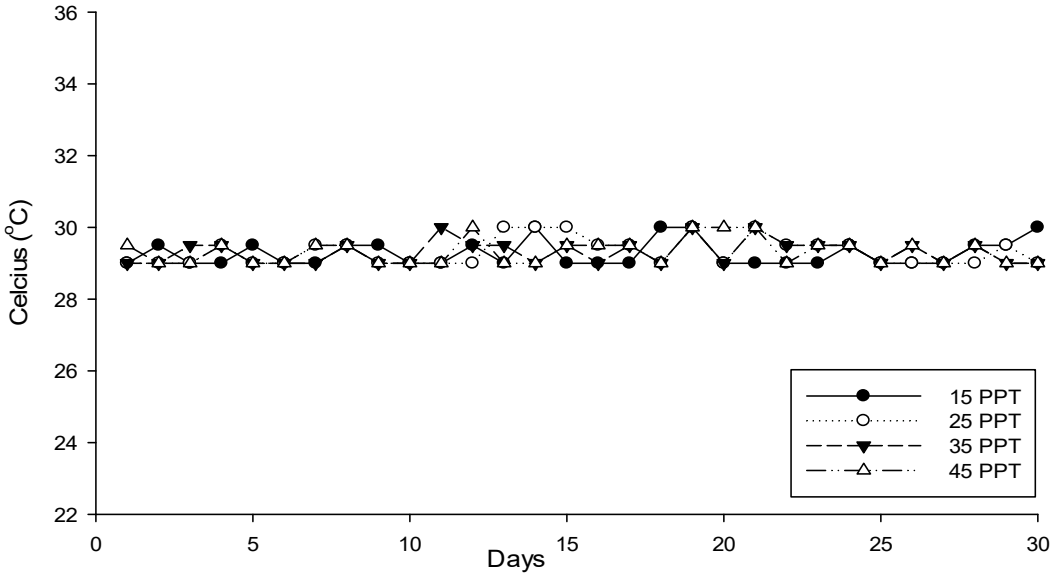

Figure 4: *Daily temperature during culture*

Based on the results of observations during culture (Figure 4), the daily temperature ranges from 29-30 °C. There is no significant temperature change as long as the culture takes. According to Chaisutyakorn et.al, this species can live at a temperature of 25-40° C and its optimal growth is at 30° C [1]. The temperature during continuous culture shows that the range is still at the temperature of tolerance of growth of *C. muelleri*

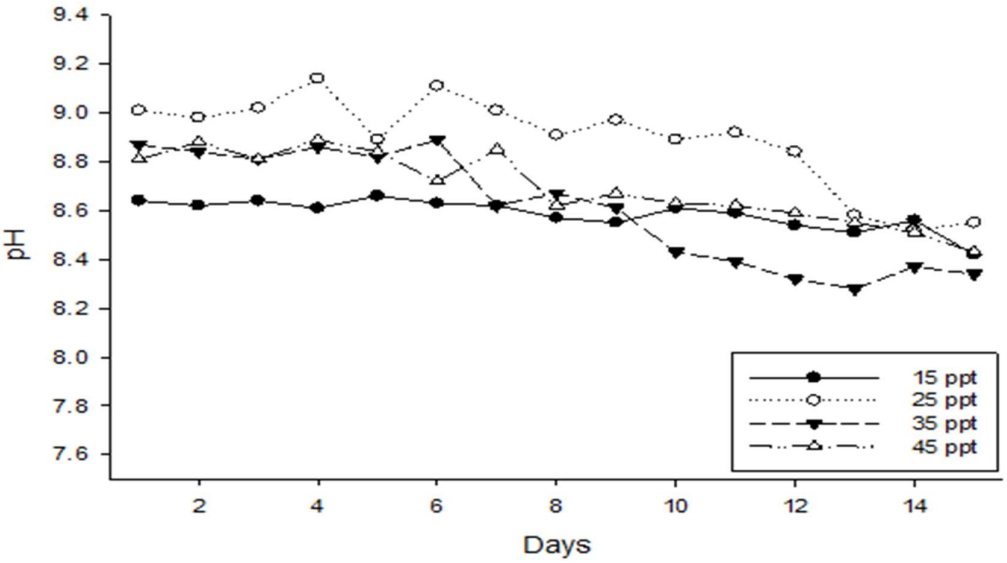

Figure 5: *Daily pH during culture*

While the results of observations on pH indicate that the pH range is in the range 8-9 (figure 5). This value is still in pH tolerance in *C. muelleri*. As explained by Jannah et.al, that the pH range for genus *Chaetoceros* is 7-9 [15]. Base on figure 5, showed there is a decrease in pH value but still within the tolerance limit. This can be caused by a decrease in photosynthetic activity. The decrease in photosynthetic activity causes CO_2 in the growth medium not to be utilized optimally so that the pH decreases slightly at the end of the culture. This reduction in photosynthetic activity is thought to be a cell response to salinity conditions.

4 Conclusion

Salinity has been known to influence the growth and content of biochemical compounds in microalgae species. This study shows the same thing, where each salinity treatment has a different effect, on growth and lipid content in each phase. The highest cell density was obtained at a treatment of 25 ppt with cell density reaching 3.80 ± 0.49 x 106 cells. ml^{-1} and a maximum growth rate of 0.36 ± 0.008 div. day^{-1}. While the lowest cell density was obtained at 45 ppt treatment with a cells density of 1.85 x 10^6 cells. $ml^{-1}$and a growth rate of 0.09 ± 0.073 div. day^{-1}. The highest lipid content was obtained in the final stationary phase, at 35 ppt treatment with lipid percentage reaching 25.37% of the total dry weight. During culture, the lipid accumulation does not occur in the exponential phase only, even when the culture reaches the stationary phase lipid accumulation still occurs. Therefore, increasing lipids using salinity in continuous photobioreactors can be applied to make lipid production effective and maintain microalgae culture longer.

5 Declarations

5.1 Study Limitation

This research did not classify the fatty acids from the total lipids obtained. This is due to the unavailability of GC-MS devices in The Marine Microbiology laboratory, Padjadjaran University.

5.2 Acknowledgments

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5.3 Funding Source

None

5.4 Competing Interests

The authors declared that no conflict of interest exist in the publication of this work.

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