Protein Production of Dunaliella sp. Cultured in Various Salt Concentrations

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Abstract: Dunaliella sp. is a potential microalga to be developed as a natural feed because it has a high protein content. This microalgae growth is influenced by salinity concentration. Salinity can also affect the biochemical composition of microalgae. This study aimed to determine Dunaliella sp. growth and protein content cultured at various salinity concentrations to select an effective salinity to produce growth and high protein content. The research output is to find the salinity so Dunaliella sp. can grow well with high protein as feed. The research method was experimental, with a completely randomized design consisting of six treatments and three repetitions. The treatments tested were salinity 0, 5, 10, 15, 20, and 25 ppt observed for nine days of culture. The main parameters measured were cell density, biomass, and protein content. Supporting parameters were temperature, pH, and light intensity. The results showed that the effective salinity to increase the cell density of Dunaliella sp. was 15 ppt. The highest peak cell density at 15 ppt salinity is 32.6067 x 103 cells/ml, and the lowest cell density at 25 ppt salinity is 23.2267 x 103 cells/ml. The effective salinity for increasing biomass is 15 ppt, and protein content is 0 ppt.

Keywords: Dunaliella sp., natural feed, protein, salinity

1. Introduction

The availability of feed in the hatchery business must be cultivated in sufficient quantities, continuously and timely. Natural feed has several advantages compared to artificial feed. The natural feed has a high nutritional, is easily digested, the movement of the feed attracts the fish, the size is relatively small so that the larvae can easily eat it, and it does not pollute the cultivation medium (Buwono *et al.*, 2019). Therefore *Dunaliella* sp. needs to be cultivated to fulfil these needs. Various modifications in culture techniques accelerate growth to get high biomass and chemical content (Ulkhaq *et al.*, 2019).

Dunaliella sp. is a green microalga that contains protein, fat, and carbohydrates (Darsi *et al.*, 2012). The presence of this microalgae in nature is the basis of the food chain because it can produce renewable resources (Husma, 2017). *Dunaliella* sp. is microalgae that have the potential to be developed as a natural feed because it is size fits the opening of the fish mouth, has a quite fast cell division, is a good source of protein, and can adapt to the entire range of salinity from 10-50 ppt (Febriani *et al.*, 2020; Novianti, 2019; Facta *et al.*, 2006; Fakhri *et al.*, 2020).

Media salinity will affect microalgae growth, development, pigment content, and chemical composition. Salinity can be a major stressor and growth inhibitor in terrestrial and aquatic biota. Extreme salinity will cause osmotic pressure or ion exchange that affects the metabolism of photosynthetic organisms (Djunaedi *et al.*, 2017). The higher salinity will slow growth, nitrate consumption, and protein synthesis (Fakhri *et al.*, 2020; Yao *et al.*, 2013).

Fakhri *et al.* (2020) stated that the optimum salinity for *Dunaliella* sp. growth and biomass production is 15 ppt. Using *Dunaliella* sp. as a natural feed makes it culture by lowering or increasing the salinity of living conditions in brackish water, which is expected to make *Dunaliella* sp. able to live in various water and produce high biomass and protein content. This study aimed to determine the cell density, biomass and protein content of *Dunaliella* sp. cultured at various concentrations of salinity so that it can be determined effective salinity to increase the growth and protein content of *Dunaliella* sp.

2. Methods

This research was conducted for five months, starting October 2022 until February, in the Aquatic Biology Laboratory, Faculty of Biology, Jenderal Soedirman University. This study uses an experimental method with six treatments, namely variations in salinity concentration including 0, 5, 10, 15, 20, and 25 ppt. Growth observations were seen from cell density. Protein content was analyzed using the Kjeldahl method. The main parameters measured were cell density, biomass and protein content of *Dunaliella* sp. Supporting parameters measured at the culture beginning and end include temperature, pH of the culture medium, and light intensity.

The materials used in this study include *Dunaliella* sp. from BBPBAP Jepara with brackish conditions, distilled water, Growmore 32-10-10, Natrium chloride (NaCl), or table salt, Chlorine ($C_{20}H_{16}N_4$), Na-thiosulfate ($Na_2S_2O_3$), alcohol 70% for sterilization, soap, and water. The destruction solution includes Selenium (Se) as a catalyst and sulfuric acid (H_2SO_4). Distillation solution includes Natrium hydroxide 30% (NaOH), boric acid 4% (H_3BO_3), and Tashiro indicator. The titration solution is hydrochloric acid 0,01 N (HCl).

The tools used in this study include culture equipment (aerator/air pump, 1000 ml culture bottle, aeration hose, aeration stone, hose connection), refractometer, pH meter, thermometer, lux meter, lamp, light microscope, object glass, cover glass, hand counter, analytical balance, drop pipette, beaker glass, plankton net, Kjeldahl flask, deconstruction device, steam distillation device, titration device, funnel, filter paper, filter holder, vacuum filter, scissors, aluminium foil, tissue, brush, washing sponge and label.

The method includes sterilization of tools, propagation of microalgae seeds, making culture media with various salinity concentrations, calculating the initial density of microalgae, and carrying out culture. The sterilization of culture equipment is done by washing the equipment washed with soap and flowing water, then soaked in a chlorine solution (150 mg/L) for 12-24 hours. After that, the tool was soaked again with 50 mg/L Na-thiosulfate solutions for 1-2 hours and rinsed with fresh water until the chlorine smell disappeared. Microalgae are propagated by mixing 30% into 70% of the growing medium.

Preparation of culture media with variations in salinity concentration was carried out by mixing 1 g/L of Growmore fertilizer with 800 ml of distilled water into 1000 ml sterilized culture bottles. Salt was added until a variation of salinity concentration of 0, 5, 10, 15, 20 and 25 ppt was obtained. The initial density calculation is done to determine the volume of seedlings stocked. The measure of seedling volume is done with the following formula:

$$V_1 = \frac{N_2 \times V_2}{N_1}$$

Description:

V_1 = Seed volume for stocking (ml)
N_1 = Initial density (cells/ml)
V_2 = Volume of media culture (ml)
N_2 = Desired seedling density (cells/ml)

Culture implementation includes cell density calculation, harvesting, biomass calculation, and protein content measurement. Calculations were made with 1 sample drop (1 ml = 20 drops). The measure was done by shifting the object glass horizontally and vertically so that all cover glass surfaces were observed. Cell density was calculated directly with a 400-times magnification light microscope. Harvesting is done with a filter tool such as plankton net no. 25. Samples were filtered with a plankton net and then filtered again with Whatman Cat filter paper No. 1441 (125 mm). The following formula calculates biomass *Dunaliella* sp.:

$$Biomassa = \frac{(B - A) \times 1000}{Volume\ sampel}$$

Description:

A = weight of filter paper
B = weight of filter paper + microalgae

Analysis of protein content using the Kjeldahl method, which consists of 3 stages, namely the deconstruction, distillation, and titration stages. The protein content is calculated by the formula (Sudarmadji *et al.*, 1996):

$$\%\ protein = \frac{(V\ HCl\ sampel - V\ HCl\ blanko) \times 14{,}007 \times fk}{W \times 1000}\ x\ 100\ \%$$

Description:

Cf = conversion factor (6,25)
W = sample weight

The cell density of *Dunaliella* sp. was analyzed using analysis of variation (ANOVA) with 95% and 99% confidence levels (Appendix 6). The results of the ANOVA test are significantly different, then continue with the LSD test (Least Significant Differences) (Appendix 7). The LSD test aims to determine the difference between the treatments. The biomass and protein content data are presented in histograms and analyzed using descriptive analysis.

3. Result and Discussion

The observation of growth is seen from the cell density along the culture of *Dunaliella* sp. on culture media with various concentrations of salinity conducted for nine. Based on the ANOVA test of cell density, *Dunaliella* sp. with different salinity concentrations showed that treatment was non-significant on days 1 and 2 ($p<0.05$). Day 5 treatment was significant ($p<0.05$), and on days 3, 4, 6, 7, 8, and 9, the treatment was very significant ($p<0.01$).

Days 1 and 2 showed non-significant ANOVA results, and this occurred because microalgae cells were experiencing a lag phase or adaptation to the growth medium. Days 3 to 9 showed significant and highly significant ANOVA results, also indicated by the cell density, which began to increase from the initial density cultured. Munir *et al.* (2017) stated that both organic and inorganic compounds or materials influence the ability of microalgae to adapt to the media as a source of nutrients and become limiting for microalgae growth.

The results of the LSD test best treatment overall is 15 ppt. LSD results of 15 ppt have the highest cell density compared to other treatments, followed by the same letter from the third to ninth days. Fakhri *et al.* (2020) state that 15 ppt is the optimum salinity for the growth of *Dunaliella* sp. by increasing the growth rate and cell density.

The number of cells decreased at the beginning of the growth of *Dunaliella* sp. (on days 1 and 2). The number of cells is less than 1x104 (initial seed culture). The decrease in the number of cells occurs because microalgae need time to adjust to the new environment, called the lag phase. The lag phase is where microalgae cells begin to adapt to a new environment so that the microalgae population does not increase in this phase. (Megawati & Damayanti, 2020). The lag phase can occur for 1-3 days (Meria *et al.*, 2021). The exponential phase occurs on days 3 to 5, with a significant and continuous increase in the number of cells due to rapid division. Megawati & Damayanti (2020) stated that the exponential phase is when microalgae cells continue to grow and divide until they reach their maximum growth.

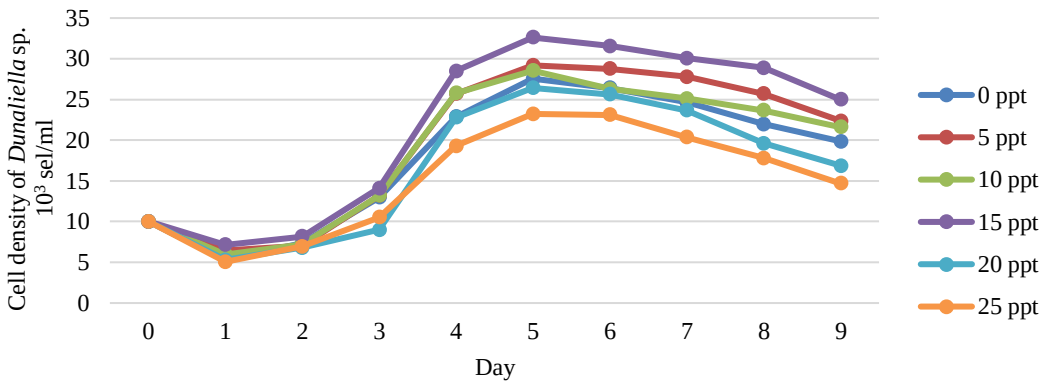

Figure 1. Cell Density of *Dunaliella* sp.

The peak of cell density occurred on the 5th day in all treatments. The highest cell density was 32.6067 x 10^3 cells/ml in 15 ppt, while the lower cell density was 23.2267 x 10^3 cells/ml in 25 ppt. At the same time, the lowest salinity that produces high growth peaks is 5 ppt salinity, with a cell density of 29.1867 x 10^3 cells/ml. On the sixth day, all treatments experienced a decreased cell density due to the limited nutrient content and volume culture media. The nutrient content decreased because no nutrient was added. Nutrient application is only done at the beginning of the culture. Megawati & Damayanti (2020) state that an increase in the microalgae population in the exponential phase causes nutrients to decrease very quickly and affects the decrease in growth rate.

Salinity in the culture medium affects the growth of *Dunaliella* sp. This study showed that too high or low salinity would decrease growth. Zainuddin *et al.* (2017) state that the lower and higher the salinity of the optimum salinity, the lower the growth rate. According to Lukitasari (2015), low salinity will cause microalgae cells to experience a hypotonic condition, namely, the condition of the solution in the environment having a lower concentration than the liquid in microalgae cells, so that cells will swell and burst. In contrast, high salinity will cause microalgae cells to experience a hypertonic condition, where the condition in microalgae cells so that cell shrink and shrivel. This situation affects growth, resulting in decreased growth.

In addition to salinity, other factors affect the growth of *Dunaliella* sp., such as temperature, pH and light intensity. Environmental conditions measured at the culture beginning and end are increased and decreased. This can occur due to changes in microalgae cell density. The temperature range for the growth of *Dunaliella* sp. is 20-40 ° C, pH 5.5-9.5, and light intensity 2000-8000 lux (Kawaroe *et al.*, 2010; Shabana *et al.*, 2016; Padang *et al.*, 2018). According to Nurlaili *et al.* (2015), an increase in temperature and changes in pH in the culture medium occurs due to an increase or change in the number of microalgae populations. This indicates that the environmental conditions of culture media in this study are suitable for the growth of *Dunaliella* sp. In addition, there is also competition for living space because the number of cells increases while the volume of media remains (Rusyani, 2001).

Biomass *Dunaliella* sp. in culture with different salinity shows differences in biomass in each treatment. The highest biomass obtained in the treatment of 15 ppt is 0.3875 g/L, and the lowest in the treatment of 25 ppt is 0.2395 g/L. As for salinity below and above, the optimum salinity that produces high biomass is 5 ppt with a biomass of 0.3320 g/L. Dunaliella sp. cultured at 5 ppt can produce reasonably high biomass. Djunaedi *et al.* (2017) stated that the difference in biomass is related to *Dunaliella* sp. cell size, density, and growth rate. Cell size varies following growth conditions and environmental changes such as salinity. According to Ferraris *et al.* (1986), salinity influences the regulation of internal ions to maintain the internal environment. This affects physiological processes, disrupting metabolism. Disrupted metabolism will affect the quantity and quality of biomass production.

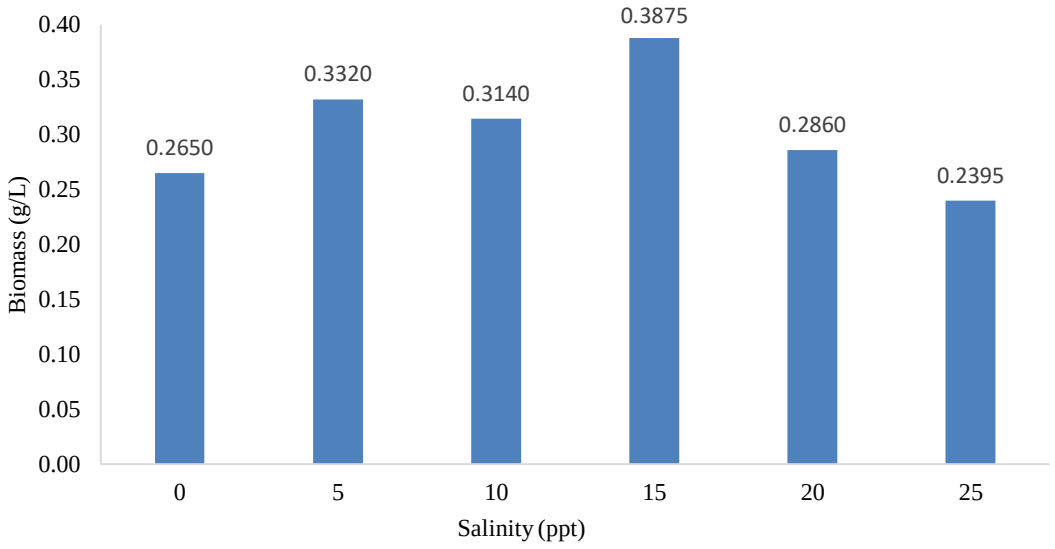

Figure 2. Biomass of *Dunaliella* sp.

The higher the salinity of the culture medium, the protein content produced by *Dunaliella* sp. decreased. The highest protein content was in the treatment of 0 ppt, which amounted to 10.087%, while the lowest was in the treatment of 25 ppt salinity, which amounted to 4.571% (Figure 4.3). The high protein content is not obtained from maximum cell density and biomass. Salinity treatment 15 ppt has high cell density and biomass, but salinity treatment 0 ppt salinity has a high protein content. The 5 ppt salinity also shows the results of a reasonably high protein content of 9.049%. According to Fabregas *et al.* (1986), protein content is not always related to cell density. Because the biochemical composition of microalgae can change depending on the environment, and protein content tends to decrease with increasing salinity.

The protein content obtained in this study was about 4,571% - 10,087%. Based on research by Tammam *et al.* (2011), *Dunaliella* sp. cultured on media with various salinity concentrations produced protein between 3,66% - 7,20%. The difference in salinity is due to differences in the quality of seeds and nutrients in the culture medium that impact *Dunaliella* sp. growth and protein content differently.

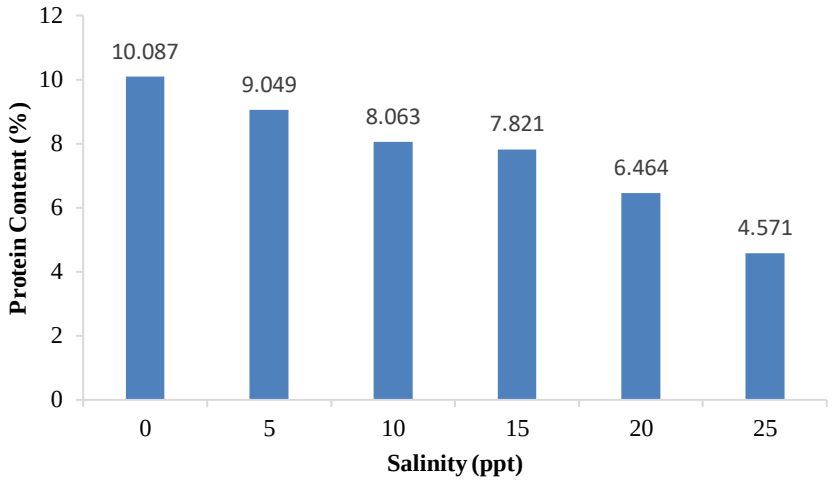

Figure 3. Protein Content of *Dunaliella* sp.

Conclusion

Salinity affects the cell density of *Dunaliella* sp., higher or lower salinity decreases the cell density of *Dunaliella* sp. Culture media with different salinity concentrations result in differences in biomass and protein content produced by *Dunaliella* sp., salinity with high cell density produces high biomass, and low salinity produces high protein content. Effective salinity to increase growth with high cell density in *Dunaliella* sp. is 15 ppt, and increasing the protein content is 0 ppt.

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