The Effect of Nitrogen and Salinity on The Content of Lipid *Dunaliella salina*

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Abstract

Microalgae are micro-sized algae plants that can usually be found in fresh and marine waters. One of the marine microalgae that has the potential to be developed is Dunaliella salina because it has a fairly high nutrient content, namely 57% carbohydrates, 32% protein, and 6% lipids. Cultivation of D. salina and other microalgae in general is very dependent on several things, including nutrient content, salinity, temperature, pH, and light intensity. The nutrient that D. salina needs in large quantities is nitrogen. Besides nitrogen, salinity is also one of the other factors that also affect D. salina because D. salina is a halophilic microalgae, namely microalgae that have the ability to survive in conditions of high salt content (20–40). This study aims to determine the effect of nitrogen and salinity on lipid D content in D. salina, as well as the best nitrogen concentration and salinity concentration that can produce the highest lipids in D. salina culture. This research method is experimental. The research design used was a randomized block design (RBD) factorial pattern. There are two factors, namely factor A, which is the concentration of salinity, namely A1 = 25 ppt salinity, A2 = 30 ppt salinity, A3 = 35 ppt salinity, and A4 = 40 ppt salinity. Factor B is the nutrient content, namely B1 = NaNo 3 100g/L (control), B2 = NaNo 3 75g/L, B3 = NaNo 3 50g/L, and B4 = NaNo 3 25g/L. The results of the data analysis study showed that the highest lipids in D. salina could be detected on the 7th day in treatment M, namely 40 ppt salinity and 25g/L nitrogen concentration, which had the highest lipid yield of 0.7720%. Under low nitrogen conditions, microalgae can produce energy reserves such as lipids. The lowest lipid on day 7 was found in treatment D with a salinity of 25 ppt and a nitrogen concentration of 100 g/L, which was obtained at 0.3180%. Under high nitrogen conditions, microalgae produce few energy-reserve compounds such as lipids.

Keywords: *Dunaliella salina,* Microalgae, Lipid, Salinity, Nitrogen

Introduction

Microalgae are micro-sized algae plants that can usually be found in fresh and marine waters (Assadad et al., 2010). One of the marine microalgae that has the potential to be developed is Dunaliella salina because it has a fairly high nutrient content, namely 57% carbohydrates, 32% protein, and 6% lipids (Hasanuddin, 2012).

The use of lipids in microalgae has previously been studied and applied in several fields. In the field of aquaculture, the lipid content in microalgae can be used as a natural feed. In the lipid industry, microalgae are used as an alternative biodiesel feedstock because the lipid content in microalgae has the same characteristics as vegetable oils (Elystia et al., 2019). Cultivation of D. salina and other microalgae in general is very dependent on several things, including nutrient content, salinity, temperature, pH, and light intensity. An increase in lipid content can occur in conditions of nutrient deficiency. This is due to the low rate of production of cell components, which causes cell division to be inhibited, so that microalgae produce lipids as food reserves.

Salinity is another factor that also affects D. salina because D. salina is a halophilic microalgae, namely microalgae that have the ability to live at high salt levels of 20–40, so it is closely related to salinity (Smith, 2010). Salinity is a factor that can affect several biochemical and physiological mechanisms, such as lipids and growth.

Research on the effect of salinity on lipids in microalgae has been done before. Febriana et al. (2013) conducted a study on the effect of salinity on the lipid content of the Botryococcus brauni microalgae and found

that the higher the salinity in the biomass culture medium, the lower the culture, but the lipid productivity increases. Arihanda et al. (2019) conducted a study on the total lipid levels of the microalgae Nannochloropsis oculata based on differences in salinity of 31–35 ppt and light intensity of 500–3000 lux. It survives and has a content of 66.5767 ± 1.5257 mg/g.

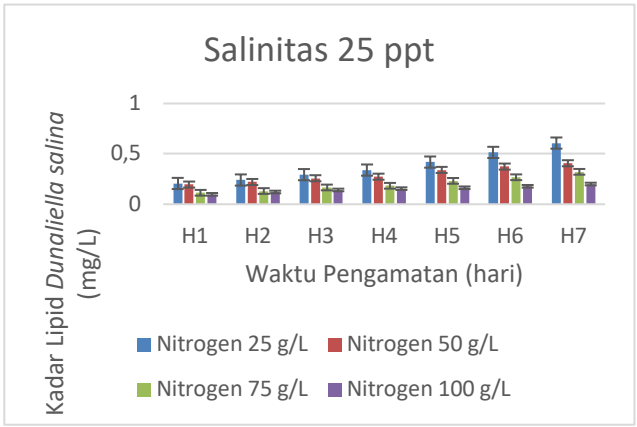
Methods

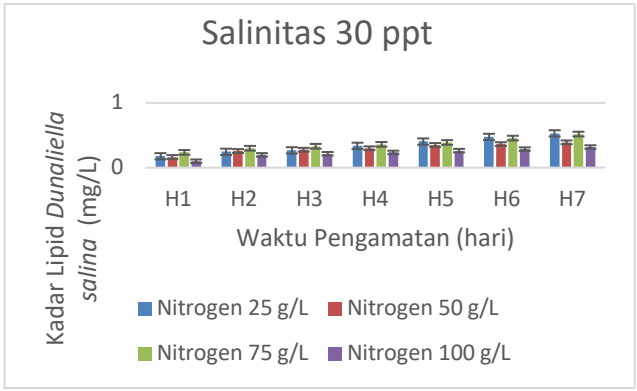
This research was conducted in July 2022–August 2022 using the secondary data collection method at the Anatomy and Aquaculture Laboratory and continued with lipid extraction at the Microbiology Laboratory, Faculty of Fisheries and Marine, Airlangga University, Surabaya.

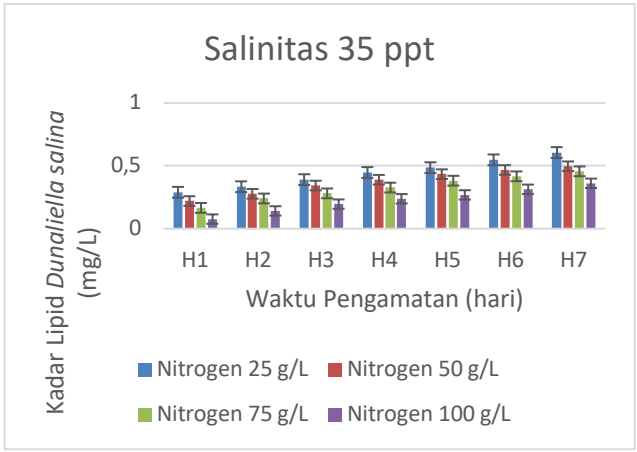
The equipment needed in the study were 16 aquariums or jars with a volume of 3 liters, a pH pen, a pH meter, a DO meter (YSI 550A), an aeration hose, a CO2 hose, a refractometer microscope, a petri dish, an LP20 aerator, stationery, an ultrasound cleaner (Power Sonic 405), a thermometer, a 50 ml burette, a stative, a clamp, a burette tap, a glass funnel, 210 gram analytical balance, spray bottle, rotary evaporator (VRT-PRO series), rotary evaporator (VRT-PRO series), rotary evaporator (VRT-PRO series), rotary evaporator (VRT-PRO Hot plate MS-H280-PRO, oven, 50 ml Erlenmeyer, dropper pipette, 10 ml volume pipette, and suction cup (bulb) The research materials used consisted of Dunaliella salina inoculants. The materials used during the study consisted of Dunaliella salina inoculant obtained from the Brackish Water Aquaculture Fisheries Center (BPBAP) Situbondo, HCL 5% Sip Chemicals, sea water, Walne fertilizer, gas CO2, Soghieklin 60 ppm chlorine, distilled water, label paper, 200 ml Dunaliella salina suspension, NaNO3 Sap Chemicals, methanol absolute Smart Lab, Whatman filter paper no. 41, and NaCl Koy salt.

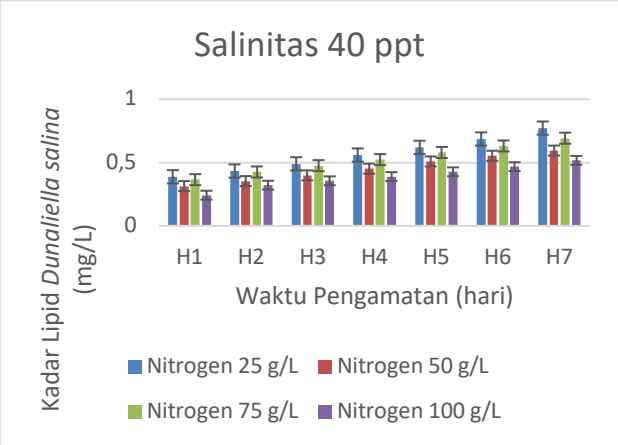
This research method is experimental. The research design used was a randomized block design (RBD) factorial pattern. There are two factors, namely factor A, which is the concentration of salinity, namely A1 = 25 ppt salinity, A2 = 30 ppt salinity, A3 = 35 ppt salinity, and A4 = 40 ppt salinity. Factor B is the nutrient content, namely B1 = NaNo 3 100g/L (control), B2 = NaNo 3 75g/L, B3 = NaNo 3 50g/L, and B4 = NaNo 3 25g/L.

Results and Discussion









Observations from the research that has been done include data on the chlorophyll-a content of Dunaliella salina. Measured once a day from day 0 to day 7. Based on the graph of the lipid content of D. salina, it can be seen that at a salinity of 40 ppt and a nitrogen concentration of 25 g/L, it has the highest lipid content obtained on the 7th day of 0.772 mg/mL. The lowest lipid content at 25 ppt salinity with a nitrogen concentration of 100 g/L had a lipid content obtained on the 7th day of 0.201 mg/mL

Based on the results of the analysis using the Anova Test followed by Duncan's Test in studies with different nitrogen concentrations and salinities that affected the growth of Dunaliella salina, Nitrogen and salinity are two of the factors that affect the growth of microalgae. This is because nitrogen and salinity have a role that is related to each other; high and low salinity can cause a lower growth rate because nitrogen is low and microalgae lack organic matter needed in the process of photosynthesis (Komarawidjaja, 2016). According to Rusyani (2001), the factor that affects the growth rate is the nutrient content contained in the culture medium because the nutrient content in the culture medium is limited. Nutrient content greatly affects microalgae, especially nitrogen, because it is the main element needed to support microalgae cell elements and form important compounds in cells (Wijaya, 2006).

The highest lipid content in this study was 40 ppt salinity plus 25g/L nitrogen, with a lipid content of 0.772 mg/l. Under low nitrogen conditions, microalgae can produce energy-reserving compounds such as lipids (Arief, 2017). Low nitrogen can also reduce lipid productivity because when algae culture is deficient in nitrogen, it can reduce growth rates. Nitrogen and salinity are related; if nitrogen is low and salinity is high, then the lipid content is high. D. salina is able to tolerate extreme salinity by forming active organic substances in cells (Soeder and Stengel, 1974). At high salinity, Dunaliella salina is able to produce reserves of compounds such as lipids (Nisa et al. 2020). The lowest lipid content in this study was a salinity of 25 ppt + 100g/L nitrogen with a lipid content of 0.2006 mg/l. Under high nitrogen conditions, microalgae produce few energy-reserve compounds such as lipids (Arief, 2017). High nitrogen and low salinity can result in low lipid content.

The acidity (pH) of the environment determines the solubility and availability of mineral ions, which can affect the availability of nutrients in the process of photosynthesis. Sharp changes in pH affect enzyme activity and prevent the process of photosynthesis in microalgae (Prihantini et al. 2010). Based on the measurement results obtained, pH ranges from 7.50 to 8.20. According to Jati et al. (2012), the optimal pH range for environmental conditions of phytoplankton is 7.8–8.9.

Conclusion

The conclusions of this study are:

1. Differences in nitrogen concentrations and salinity concentrations can affect growth in D. salina cultures.
2. The best concentration of nitrogen and salinity for obtaining high lipids is a salinity of 40 ppt, and a nitrogen concentration of 25g/L has the highest lipid obtained at 0.772 mg/l.

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