Lipid Contain of Three Microalgae on Culture with Different pH and Salinity

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Abstract. Three species of microalgae were used in this study, namely *Nitzschia* sp., *Porphyridium* sp., and *Tetraselmis* sp., which collected from the Lampung Agency of Maricultures – Ministry of Coastal and Marine Affairs. They were cultured in room temperature with 12:12 day light and given different salinities and pH for treatment groups with complete randomized design. *Nitzschia* sp. and *Porphyridium* sp. were treated within factorial design 2x2, namely 20 and 40 ppt in salinities and 5 and 10 in pH, while the *Tetraselmis* sp. was given with factorial 3x3, namely 10, 15, 20 ppt of salinities and 5, 8, 9.5 in pH. All the cultures for 7 days were replicated 3 times. Analysis of variance followed by LSD at 5% level of significant was applied to analyse the data on specific growth rate and total lipid contains. The results indicated that highest growth rate of *Nitzschia* sp. was in 20 ppt of salinity and at pH of 10, while *Porphyridium* sp. was in 40 ppt salinity and at pH of 5. But the highest lipid content of *Nitzschia* sp. was in 40 ppt of salinity with 5 pH, and *Porphyridium* sp. was in 40 ppt of salinity and 5 in pH. *Tetraselmis* sp. had the highest growth rate in 20 ppt of salinity and at pH of 9.5, but the highest total lipid of it was in 10 ppt of salinity with 9.5 pH.

1. Introduction

Indonesian waters have an area of 3.1 million square kilometers with a coastline of 80,791 kilometers [1] . Marine resources found in Indonesian waters are very abundant and diverse. Microalgae, including one of the marine resources owned by Indonesia, is possible to be used for the development of industrial use of microalgae [2]. Microalgae have an important role as a primary producer which in a food chain is used as the first level, because it has the ability to photosynthesize as a high-level plant by absorbing sunlight, water, and carbon dioxide which is converted into energy [3]. According to Isnansetyo and Kurniastuty, [4] microalgae are often used as natural feed, namely *Nitzschia* sp. and *Tetraselmis* sp. *Teraselmis* sp. consumed by shrimp larvae, ornamental fish, and sea cucumber larvae, while *Nitzschia* sp. consumed by fish, bivalves, and crustaceans. Besides, microalgae *Porphyridium* sp. can also be used as natural food by marine organisms.

The potential of microalgae *Nitzschia* sp., *Porphyridium* sp. and *Tetraselmis* sp., besides as a natural food is can be developed as an alternative source of raw materials of biofuels to replace energy from fossil fuels by creating an alternative source of renewable energy sources. These microalgae has a fairly high lipid content as expressed by Chisti, [5] that *Tetraselmis* sp. has 15-23% lipid content, *Nitzschia* sp. with amount of 45-47% [6], and *Porphyridium* sp. is 14% [7]. Lipids are organic compounds that found in nature and heterogeneous. Lipids are soluble in non-polar organic solvents (other are pentane, benzene, diethyl ether, alcohol, and chloroform), but difficult to dissolve in water.

The growth of microalgae is generally influenced by environmental conditions such as salinity and pH. Microalgae will accumulate greater lipids when the environmental conditions are abnormal or environmental stress [3]. Widianingsih *et al.*, [8] states that microalgae adaptation will tend not to spend a lot of energy, because they survive by using lipids in their body. Microalgae carrying out photosynthesis using carbon dioxide (CO_2) and its accumulation results are inside carbohydrates and lipids. Based on these explanations, it is necessary to do research on the ability to produce total lipids of microalgae *Nitzschia* sp., *Porphyridium sp* , and *Tetraselmis* sp . on giving osmotic stress in different pH and salinity.

2. Material and Methods

2.1 Place and Time of Research

This research was conducted from November 2018 to February 2019, at the Laboratory of Molecular Biology, Department of Biology, Faculty of Mathematics and Sciences, University of Lampung.

2.2 Stages of Research

The research was conducted on a laboratory scale with an experiment method. The method that used in this research was Completely Randomized Design (CRD), for factorial 2x2 of mikroalga *Nitzschia* sp. and *Porphyridium* sp., with salinity 20 and 40 ppt with pH 5 and 10. Each type of microalgae was using 4 treatments with 3 repetitions. While the factorial of *Tetraselmis* sp. microalgae are 3x3 with salinity 10, 15, and 20 ppt with pH 5, 8, and 9.5 using 9 treatments and 3 repetitions.

The culture process of microalgae in a 3 liter glass culture bottle. Microalgae that contained in bottles are given 28 watts or 8.400 lux light bulbs as many as five pieces like the schematic design (Figure 1). The bottle is also equipped with an aerator to help the growth of microalgae.

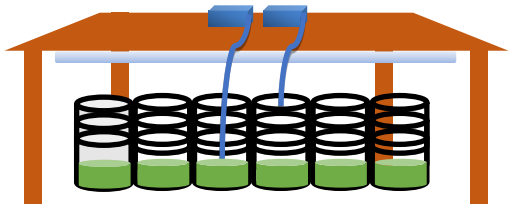

Figure 1 . Illustration of Research Design

2.2.1 Preparation media and culture sites of microalgae

Sterilized seawater using UV sterilizer then ozonated for 15 minutes at the Center for Marine Cultivation Development called BBPBL. Then the sea water treated with different pH and salinity based on the previous research design.

2.2.2 Nutrients of microalgae

Nutrition or feed given is Conwy pro analyst (PA) fertilizer. Conwy PA fertilizer consists of macro and micro elements. Macro elements consist of Na_2 EDTA (45 g), 22 $FeCl_3.6H_2O$ (1.50 g), H_3BO_3 (33.6 g), $NaH_2PO_4.2H_2O$ (20 g), $MnCl_2.4H_2O$ (0.50 g), $NaNo_3$ / KNO_3 (84.148 g / 100 g) with 100 ml aquabides or distilled water which was added with a solution of Trace Metal Solution which is a micro element that consists of $ZnCl_2$ (2.10 g), $CuSo_4.5H_2O$ (2.00 g), $CoCl_2.6H_2O$ (2.00 g), (NH_4) $6Mo7O_24.4H_2O$ (0.90 g). Conwy PA fertilizer was carried out at the beginning of the culture as much as 1 m L/L [9].

2.2.3 Culture of *Nitzschia* sp. , *Porphyridium* sp. , and *Tetraselmis* sp.

The microalgae that obtained from BBPBL were taken as much as 125 mL per sample, then filtered using filter paper. The filter of microalgae was transferred into a culture bottle which had been filled with 500 mL of sterile sea water through a boiling process and giving treatment in the form of different salinity and pH . The ratio of microalgae density to sea water is 1: 4 [9] . The maintenance process is carried out for 7 days until the peak phase is harvested and a total lipid level is tested at the stationary phase.

2.2.4 Population Density of *Nitzschia* sp., *Porphyridium* sp., and *Tetraselmis* sp.

The cell density of microalgae populations is calculated every day using Haemocytometer on a microscope with a handcounter. As much as 1 mL of sample is taken every day using a dropper pipette and then transferred into a culture bottle. Adding 2-3 drops of formaldehyde is used to facilitate the observation of microalgae. As for the cell density formula according to Mudjiman [10] are as follows:

$$T = N \times 25 \times 10^4 \ (\Sigma \text{ Sel/mL})$$

Information :
T : Cell Density
N : Average number of cells (Number of 5 boxes cells / number of boxes (5))

2.2.5 Growth rate of *Nitzschia* sp., *Porphyridium* sp., and *Tetraselmis* sp.

Microalgae growth rates can be calculated using formulas according to Hirata *et al.* [11] namely:

$$K = \frac{\log(\frac{Nt}{N0})}{Tt - T0} \times 3.22$$

Information :
K : Population growth rate
3.22 : Constants

N0 : Initial microalgae density
Nt : Density of microalgae at time t
T0 : Initial time
Tt : Time of observation t

2.2.6 Analylis of total lipid levels microalgae *Nitzschia* sp., *Porphyridium* sp., and *Tetraselmis* sp.

Analysis of total lipid levels was taken from modification method of Bligh and Dyer [12]. Mikroalga *Nitzschia* sp., *Porphyridium* sp., and *Tetraselmis* sp. harvested at the stationary phase or on the 8th day. The microalgae are deposited with NaOH 1 gr/L for 24 hours. The next step is filtering the microalgae using satin fabric so that obtained in the form of a paste. The next process is extraction. The next step is to know the lipid content using methanol and chloroform with a ratio of 1: 1 or (3 mL: 3 mL) and then homogenize using vortex for about 1 minute to form 2 phases (above clear and cloudy bottom). Calculation of the percentage of total lipid dry weight as follows:

$$\% \text{ Total Lipids} = \frac{(A-B)}{C} \text{ x } 100$$

Information:
A : Weight of cup + weight of lipid after extraction (grams)
B : Weight of the cup before extraction (grams)
C : Wet sample weight (grams)

2.2.7 Data analysis of microalgae

Population density was analyzed by one-way ANOVA to determine differences in each treatment. The Smallest Significant Difference Test (LSD) was carried out after obtaining the results of variance analysis, if the results were significantly different between treatments. Growth rates and total lipid levels were analyzed descriptively. Microalgae density data were analyzed using a transformation formula according to Fowler *et al.*, [13].

$$X = \frac{Tn-T0}{T0} \text{x } 100\%$$

Information:
T0 : 0-day initial cell cell culture
Tn : nth day cell density
X : Increase / Decrease population of microalgae (%)

3. Results and Discussion

3.1 Microalgae density of cells *Nitzschia* sp., *Porphyridium* sp., and *Tetraselmis* sp.

The following data are density of *Nitzchia* sp. cells on differences in salinity and pH treatment for 7 days.

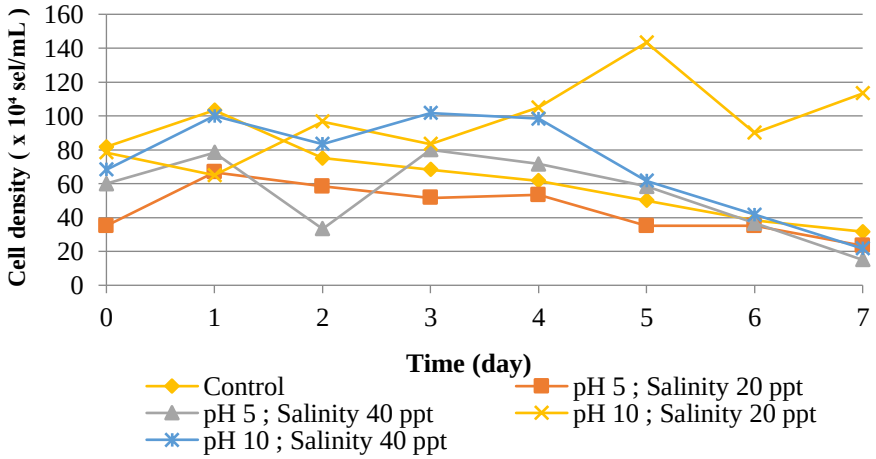

Figure 2. Cell density of *Nitzschia* sp.

Data on density of *Nitzschia* sp. cell (Figure 2) is highest in pH 10 and salinity of 20 ppt is 143.33 x 10^4 cells/mL on the fifth day, while the lowest sample is on the seventh day of the pH 5 and salinity 40 is 15 x 10^4 sel/mL. The differences sampel on days 5.6 and 7 treatment. On day 5, the sample increase cell density as big as 143.33 x 10^4 cells/mL from 105 x 10^4 s el / mL on day 4, while the other samples decreased the cell density. Then on day 6 the cell density decreases to 90 x 10^4 cells/mL. According to Tri *et al.,* [14] the journal stated that the increase could be influenced by the nutritional content contained in the media and environmental factors. High nutrient content will make the nutrients of microalgae fulfilled.

Table 1 . Growth population percentage of *Nitzschia* sp.

Day	Average (%) ± Std. Error				
	Nitzschia sp.				
	Control	**PN1SN1**	**PN1SN2**	**PN2SN1**	**PN2SN2**
	pH 7.7 & Salinity 40.2 ppt	**pH 5 & Salinity 20 ppt**	**pH 5 & Salinity 40 ppt**	**pH 10 & Salinity 20 ppt**	**pH 10 & Salinity 40 ppt**
1	7.01 ± 49.90	**84.72** ± 44.77	50.31 ± 62.44	-6.49 ± 29.67	66.80 ± 103.03
2	3.07 ± 28.49	75.93 ± 28.88	-37.72 ± 25.75	31.39 ± 50.90	27.46 ± 29.62
3	-7.11 ± 21.79	60.19 ± 36.16	64.30 ± 81.65	-5.79 ± 26.12	57.36 ± 40.82
4	-13.14 ± 27.23	72.69 ± 54.80	31.24 ± 26.87	47.92 ± 29.55	46.35 ± 40.77
5	-35.95 ± 18.02	19.91 ± 61.35	5.93 ± 35.88	82.26 ± 22.50	-6.14 ± 19.07
6	-47.97 ± 12.79	-15.74 ± 47.31	-40.55 ± 4.74	25.29 ± 45.47	-38.37 ± 16.37
7	**-55.37 ± 13.75 [b]**	**-47.22 ± 40.92 [b]**	**-77.94 ± 11.98 [b]**	**70.41 ± 54.78 [a]**	**-66.40 ± 10:58 [b]**

Description: Different letter notations state of significant differences

Based on the transformation of data on addition or decrease of population with ANOVA test there were significant results in the seventh day sample . Samples with a pH of 5 & salinity of 20 ppt on day 1 is different from other samples because the average population growth is 84.72%. This figure is among the highest among others. According to the Kawaroe study *at al.* [3] states that on the 4th to 5th day of cultivation, microalgae are in the exponential final phase and enter the stationary phase so that growth is abundant. The statement is in accordance with Table 1 which shows that the 5th day experienced an increase in population growth compared to the previous day, which was 5.93% on the 4th day and then 82.26% on the 5th day.

The following are data on the density of *Porphyridium* sp. on differences in salinity and pH cultured for 7 days.

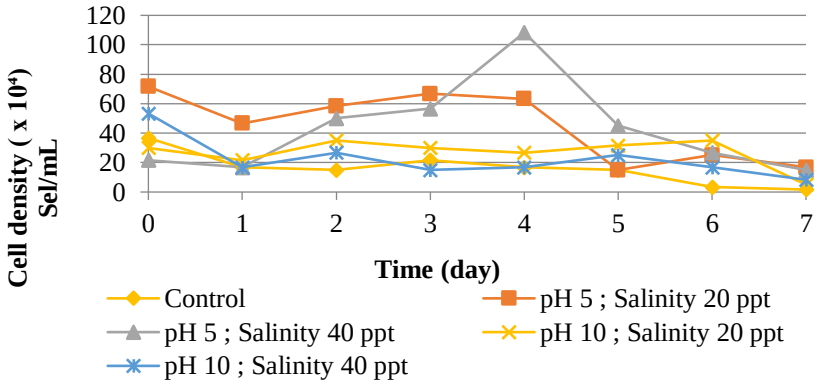

Figure 3. Cell density of *Porphyridium* sp.

Porphyridium sp. Cell density the highest in Figure 3 is in a pH sample of 5& salinity of 40 ppt of 108.33 x 10^4 s el / mL on the fifth day , while the lowest sample was on the sixth day of the Control sample of 1.67 x 10^4 sel/ mL. Based these graphs, the sample cell density began to increase on day 4 to 5, and then decreased at the 6th and 7th culture. Allegedly on the 4th and 5th day, cells are in the exponential phase, namely cell growth has increased because cells can adapt well. When experiencing an exponential phase, microalgae cells are actively reproducing by cleavage [15]. On the 6th and 7th day the cell experienced a decrease because on that day, the cell was in the stationary phase, namely the equality between the growth rate and the death rate.

Table 2 . Growth population percentage of microalgae *Porphyridium* sp.

Day	Average (%) ± Std. Error				
	Porphyridium sp .				
	Control	PP1SP1	PP1SP2	PP2SP1	PP2SP2
	pH 7.8 & Salinity 39.8 ppt	pH 5 & Salinity 20 ppt	pH 5 & Salinity 40 ppt	pH 10 &Salinity 20 ppt	pH 10 & Salinity 40 ppt
1	-48.89 ± 24.75	-34.92 ± 8.29	-5.56 ± 53.00	-33.33 ± 16.67	-67.96 ± 6.22
2	-58.89 ± 4.84	-18.25 ± 16.28	138.89 ± 105.56	19.44 ± 32.75	-50.28 ± 8.61
3	**-38.89 ± 5.56 ^{bc}**	**-6.98 ± 8.25 ^{bc}**	**150.00 ± 28.87 ^{a}**	**13.89 ± 36.11 ^{b}**	**-69.26 ± 9.65 ^{c}**
4	**-53.33 ± 10.19 ^{b}**	**-12.06 ± 10.23 ^{b}**	**441.67 ± 115.57 ^{a}**	**-4.17 ± 18.16 ^{b}**	**-67.96 ± 6.22 ^{b}**
5	**-56.67 ± 6.67 ^{c}**	**79.05 ± 0.48 ^{bc}**	**113.89 ± 80.56 ^{ab}**	**20.83 ± 42.29 ^{a}**	**-50.00 ± 9.62 ^{c}**
6	**-88.89 ± 5.56 ^{b}**	**-64.76 ± 11.23 ^{b}**	**19.44 ± 10.01 ^{a}**	**5.56 ± 29.40 ^{a}**	**-67.50 ± 3.15 ^{b}**
7	-94.44 ± 5.56	-76.98 ± 8.05	-16.67 ± 60.09	-83.33 ± 8.33	-86.94 ± 7.70

Description: Different letter notations state of significant differences

Based on the ANOVA test, there were significant results for the 3rd to 6th day samples . The highest percentage was found on day 4 of the sample pH 5, salinity 40 ppt in the amount 441.67%.

The following data are the density of microalgae *Tetraselmis* sp. transformation data from the increase or decrease in the growth population of the treatment.

Table 3. Addition or decrease percentage population of *Tetraselmis* sp. in different salinity and pH

Day	Average ± Standard error								
	10 ppt & pH 5 (S1P1)	**10 ppt & pH 8 (S1P2)**	**10 ppt & pH 9.5 (S1P3)**	**15 ppt & pH 5 (S2P1)**	**15 ppt & pH 8 (S2P2)**	**15 ppt & pH 9.5 (S2P3)**	**20 ppt & pH 5 (S3P1)**	**20 ppt & pH 8 (S3P2)**	**20 ppt & pH 9.5 (S3P3)**
1	(-64.95) ± 19.32	(-25.71) ± 16.74	(-44.35) ± 5.64	(-21.81) ± 13.15	25.71 ± 62.25	(-28.04) ± 39.58	58.33 ± 96.10	(-61.50) ± 15.21	92.85 ± 92.85
2	(-44.55) ± 25.27	(-0.95) ± 31.18	64.53 ± 75.14	53.93 ± 47.34	588.57 ± 605.71	152.38 ± 103.53	158.33 ± 36.32	(-40.87 ± 20.50	292.85 ± 146.55
3	76.38 ± 68.22	68.57 ± 57.87	0.09 ± 30.27	170.30 ± 74.89	124.76 ± 112.62	53.70 ± 75.24	266.66 ± 117.55	32.93 ± 9.33	547.61 ± 326.42
4	187.82 ± 75.02	514.28 ± 253.08	236.75 ± 141.97	455.15 ± 206.14	430.95 ± 284.53	324.07 ± 213.05	925.00 ± 198.43	155.55 ± 129.48	821.42 ± 356.88
5	232.79 ± 118.24	342.85 ± 182.38	299.16 ± 193.00	520.00 ± 190.78	488.57 ± 333.32	535.18 ± 208.02	983.33 ± 372.30	154.36 ± 101.51	1128.57 ± 583.89
6	93.16 ± 80.48	379.04 ± 140.95	400.18 ± 205.39	292.12 ± 96.49	1443.33 ± 1079.60	348.94 ± 132.39	491.66 ± 122.75	266.26 ± 167.50	840.47 ± 412.27
7	**200.64 ± 13.88 ^{c}**	**220.95 ± 12.38 ^{c}**	**658.33 ± 87.00 ^{abc}**	**603.03 ± 205.25 ^{bc}**	**837.61 ± 344.39 ^{abc}**	**388.35 ± 255.88 ^{bc}**	**1158.33 ± 297.32 ^{ab}**	**271.42 ± 143.80 ^{c}**	**1426.19 ± 523.46 ^{a}**

Description: The different superscript letters indicates difference value between treatments in the BNT test with a significance level (α) = 5%

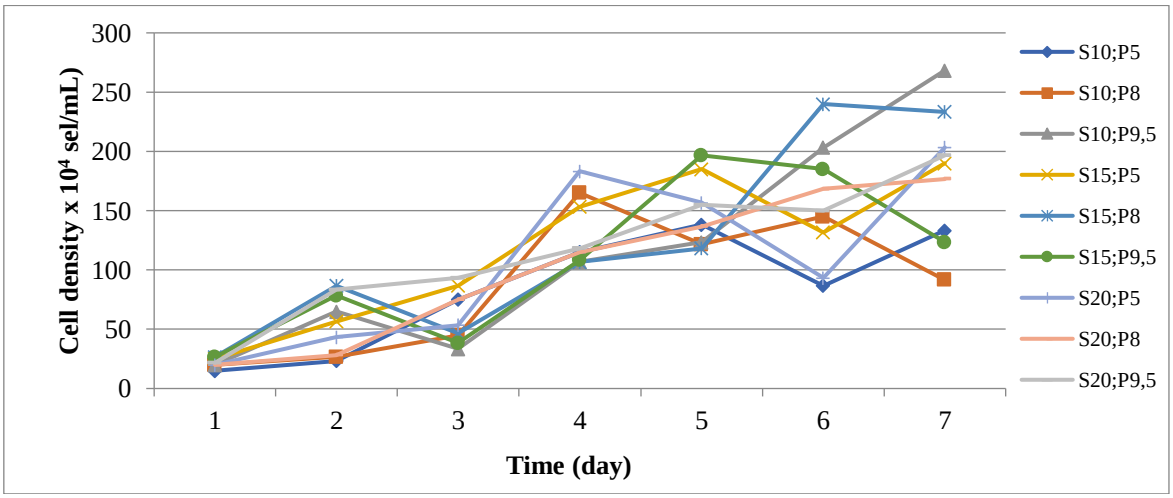

Figure 4 . Cell density of *Tetraselmis* sp. at different salinity and pH

Based on the transformation, the data obtained the percentage of addition or decrease from *Tetraselmis* sp. microalgae from highest salinity treatment 20 ppt & pH of 9.5 was 1426.19% with cell density 196.67 x 10^{4} cells/mL (Figure 4). While the lowest percentage of salinity treatment is 10 ppt & pH 5 is 200.33% with cell density 133.33 x 10^{4} cells/mL (Figure 4). Based on literature, *Tetraselmis* sp. cells more tolerant at the condition from the salinity of 20 ppt, which according to Ningsih *et al.*[16] microalgae *Tetraselmis* sp. has the highest percentage from increase the population at salinity of 20 ppt with cell density of 677.78 x 10^{4} cells/mL. Most of the microalgae tolerate the conditions of environmental changes such as salinity with a very large range and most species of microalgae well perform growth in salinity which is slightly lower than the condition of salinity in its natural habitat [17].

In addition to salinity, acidity (pH) also affects the growth of microalgae. The results obtained by *Tetraselmis* sp. has the highest cell density at alkaline pH (pH 9.5), presumably pH 9.5 is suitable for absorption of nutrients by microalgae *Tetraselmis* sp. and the continuation of optimum enzyme activity so that the metabolic process takes place quickly and causes cell density to increase. The pH level of the media can affect the workings of enzymes in the process of microalgae cell metabolism [18]. According to Nattasya [19], the pH level of media also determine the level from solubility and availability of mineral ions that affect microalgae cells in absorbing nutrients. While the lowest density at pH 5, the cell does not get nutrients properly because of the low pH that interferes with the solubility of mineral ions. According to Prihantini *et al*., [20] the treatment with the initial pH of medium 5, the lowest cell density caused by acidic initial pH can disrupt cell metabolism, and result in cells not optimally absorbing nutrients so that further growth will be disrupted.

3.2 Specific population growth of microalgae *Nitzschia* sp., *Porphyridium* sp., and *Tetraselmis* sp.

The following graph of the growth rate of *Nitzschia* sp.

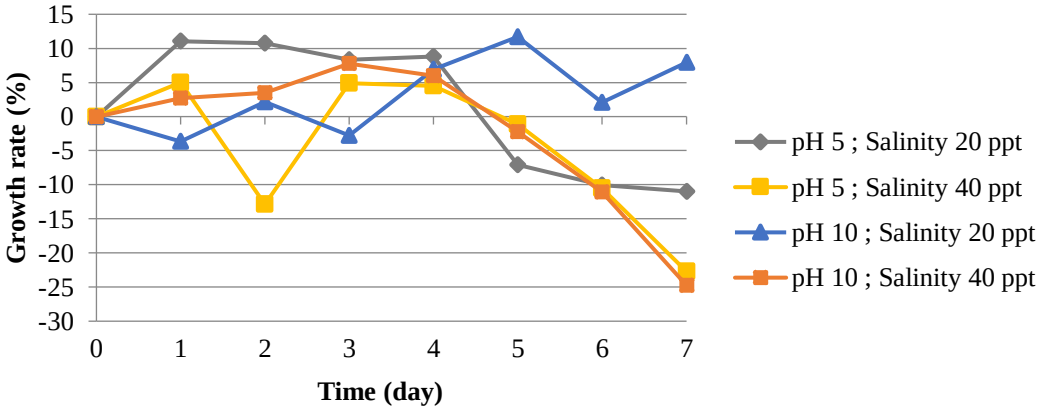

Figure 5. Growth rate of microalgae *Nitzschia* sp.

Based on the graph in Figure 5 shows that the growth rate of microalgae *Nitzschia* sp. fluctuating from day 0 to 7. The sample with the highest growth rate is on the 4th day at 11.04%, which is pH 5 & salinity of 20 ppt, while the lowest value is found in a sample of pH 10 & salinity 40 ppt decreased at 24.74% on the seventh day.

The differences salinity with their original habitat, the more severe the result adaptation process in the reproduction process and growth being disrupted. The optimum range of salinity in microalgae growth is 25-35% from dilution using fresh water [21] . Another factor also affects the growth rate of microalgae is acidity. This degree of acidity can affect the level of photosynthesis microalgae [22] and the action of enzymes in the process of metabolism of e cells [18] .

The following graph from the growth rate of *Porphyridium* sp.

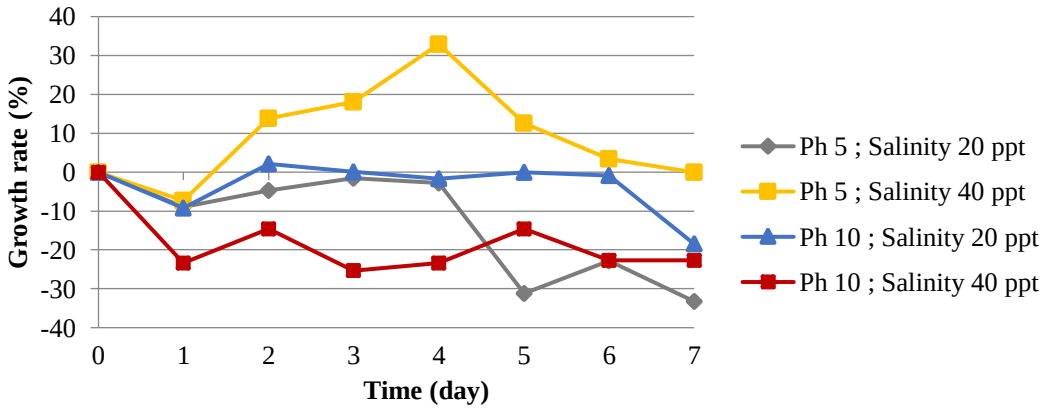

Figure 6. Growth rate microalgae of *Porphyridium* sp.

The growth rate in the chart is varied (Figure 6), the highest percentage value is 32.89% in the pH sample 5 & salinity 40 ppt in day 4 treatment, while the lowest percentage was on day 7 that decreased at 33.23% treatment from pH 5 & salinity 20 ppt. The growth rate will increase sharply on days 3, 4, 5, and 6. This is indicated by a change in the graph line that looks different from the other treatment samples. There is a graph from the sample whose growth rate is above the zero line, while the other samples are below zero. The sample is pH 5 with 40 ppt salinity, where the growth rate is above the zero line.

According to Sze [23], this is due to the degradation of chlorophyll-a so that the fixation of CO_2 becomes decreased and causes the decrease pH value. While the increasing pH is caused by photosynthesis, where free CO_2 including inorganic carbon is used as the main raw material in photosynthesis.

The following graph from the growth rate of *Tetraselmis* sp. at different pH and salinity.

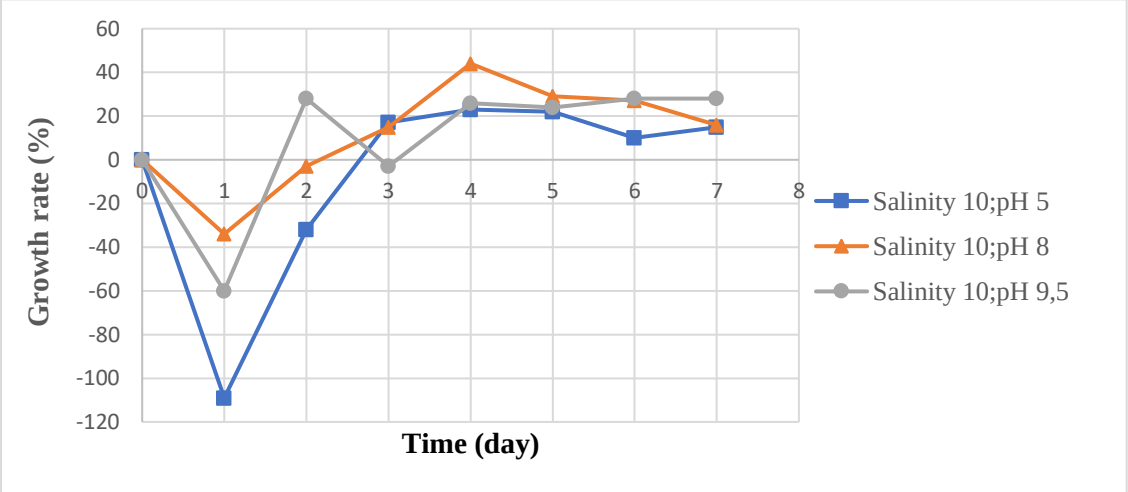

Figure 7. Specific population growth rate of *Tetraselmis* sp. salinity 10 ppt (pH 5; 8; and 9.5)

Based on the graph in Figure 7, for 10 ppt salinity & pH 5 The highest specific growth rate is found on the fourth day at 23%, while at salinity 10 ppt & pH 8 at 44%, and at salinity 10 ppt & pH 9.5 is found on the second, sixth and seventh days at 28%.

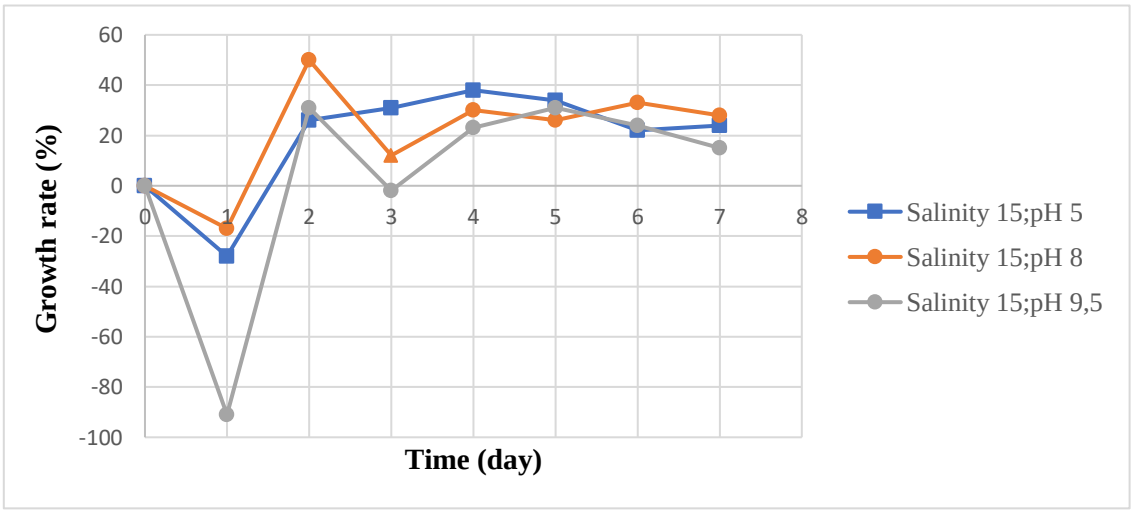

Figure 8. Specific population growth rate of *Tetraselmis* sp. at 15 ppt salinity (pH 5; 8; and 9.5)

Based on the graph in Figure 8, for 10 ppt salinity & pH 5 the highest specific growth rate is found on day four of 38%, for salinity of 15 ppt & pH 8 is on the sixth day at 33%, and for salinity 15 ppt & pH 9.5 is found on the second and fifth days at 31%.

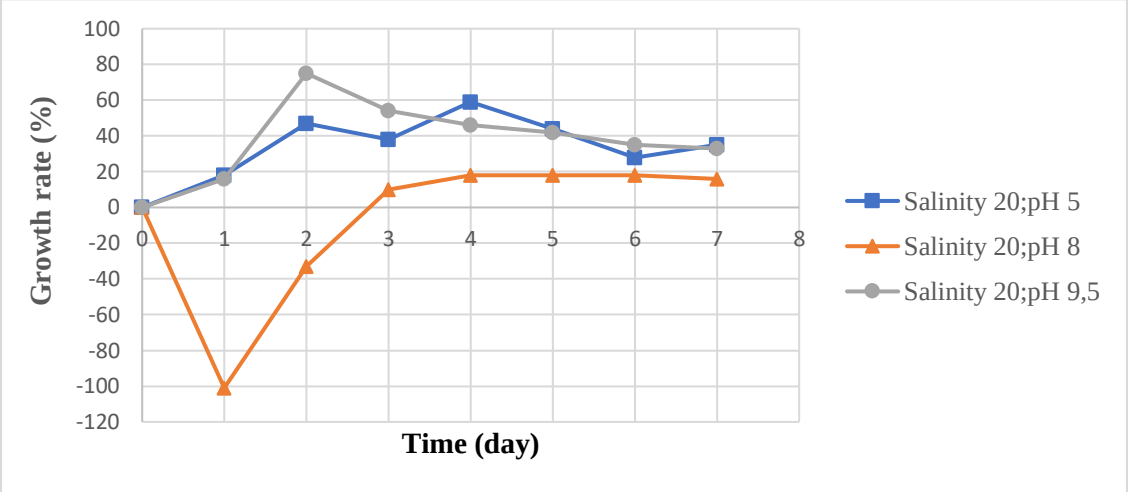

Figure 9. Specific population growth rate of *Tetraselmis* sp. at salinity 20 ppt (pH 5; 8; and 9.5)

Based on the graph in Figure 9, the highest specific growth rate for salinity is 20 ppt & pH 5 is on the fourth day 59%, for salinity 20 ppt & pH 8 is on the fourth, fifth and sixth day at 18%, and for salinity 20 ppt & pH 9.5 by 75% on the second day.

For each difference in salinity and pH, the highest specific population growth rates were found on the second and fourth days (Figures 6, 7 and 8) because cells were still in a condition with treatment media that had abundant nutrients, so that microalgae in good growth. On the second and fourth day, *Tetraselmis* sp. cells has begun to enter the exponential phase which is characterized by increasing population growth rates. Ru'yatin *et al*. [24] state that the exponential phase of the *Tetraselmis* sp. microalgae occurred on the fourth day. When experiencing this phase, microalgae cells are actively reproducing by division.

Growth of *Tetraselmis* sp. decreased the growth rate on the seventh day. The microalgae *Tetraselmis* sp. is in a stationary phase or static phase, which is characterized by a population growth rate equal to the death rate so that it is very unlikely that cells can grow. Decreased microalgae cell growth occurs due to a lack of nutrient supply so that the ability to do growth is very low [25]. This is due to the provision of fertilizers used as a source of nutrition only at the beginning of the treatment.

3.3 Total lipid levels of microalgae *Nitzschia* sp., *Porphyridium* sp., and *Tetraselmis* sp.

The lipid extraction process using the method according to Bligh and Dyer [12] obtained the lipid phase by centrifuging all microalgae samples which can be seen in Figure 1. The results of the three centrifugations were generated, namely the lipid phase, natant, and supernatant. The lipid phase that will be used to determine the lipid weight of each sample.

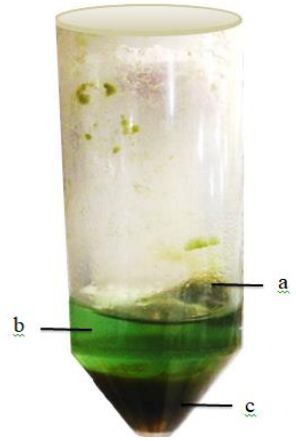

Figure 10. Microalgae pellet after centrifugation (a) natan phase, (b) lipid phase, and (c) supernatant phase.

The following are the total lipid levels of microalgae *Nitzschia* sp. , *Porphyridium* sp. , and *Tetraselmis* sp.

Table 4. Total lipid levels from three types of microalgae

Types of Microalgae	pH	Salinity (ppt)	Microalgae Wet Weight (g)	Dry Lipid Mass (g)	Total Lipids (%)	Absorbance Value *
Nitzschia sp.	Control	Control	3,000	0.045	1.50	0.044
	5	20	3,000	0.042	1.40	0.045
	5	40	3,000	0.046	**1.52**	0.040
	10	20	3,000	0.041	1.35	**0.083**
	10	40	3,000	0.025	0.83	0.033
Porphyridium sp.	Control	Control	3,000	0.022	0.73	0.042
	5	20	3,000	0.022	0.73	**0.057**
	5	40	3,000	0.034	**1.14**	0.037
	10	20	3,000	0.020	0.66	0.016
	10	40	3,000	0.021	0.70	0.051
Tetraselmis sp.	5	10	2,0016	0.0468	2.34	0.119
	8	10	2,0041	0.0973	4.86	0.059
	9.5	**10**	**2.0043**	**0.2864**	**14.29**	**0.124**
	5	15	2,0048	0.0998	4.98	0.091
	8	15	2,0020	0.0763	3.81	0.087
	9.5	15	2,0048	0.1562	7.79	0.078
	5	20	2.0049	0.0478	2.38	0.057
	8	20	2.0050	0.1213	6.05	0.085
	9.5	20	2,0054	0.1649	8.22	0.095
	Biosolar		-	-	-	0.084

* the absorbance value of a blank solution (aquades) is 0 (zero)

In Table 4, the highest total lipid microalgae *Nitzchia* sp. was found in the treatment sample pH 4 and salinity 40 ppt of 1.52%, while the lowest lipid was 0.83% in the treatment sample pH 10 and salinity 40 ppt. The highest absorbance value after Spectrophotometry test was 0.083 at the pH 10 treatment and 20 ppt salinity.

In *Porphyridium* sp. microalgae in pH 5 and salinity 20 ppt treatment, obtained a dry lipid mass of 0.022 grams which is the same as the Control sample, as well as the percentage of total lipids obtained the same sample with the Control of 0.73%, but the absorbance values of the two samples are different. The absorbance value of the sample pH 5 and salinity 20 ppt is 0.057 (highest) , while the Control sample produces an absorbance value of 0.042.

Based on Table 4 it also shows that the highest total lipid levels of *Tetraselmis* sp. microalgae was found in the salinity treatment of 10 ppt with a pH of 9.5 was 14.29%. Along with the absorbance value and dry weight obtained which was the highest value. When it compared with the other treatments namely 0.124 and lipid dry weight of 0.2864 grams. While the lowest percentage of lipid levels was found in salinity of 10 ppt with pH 5 obtained a value of 2.34%.

Can be seen in Table 6, *Tetraselmis* sp. microalgae salinity of 10 ppt, 15 ppt, and 20 ppt can produce high lipid levels. This is due to the microalgae *Tetraselmis* sp. has a hyper osmotic nature (resistant to high salinity). As research conducted by Ningsih *et al*. [16] microalgae *Tetraselmis* sp. microalgae when given a salinity of 30 ppt (normal) a lipid level of 1.26% was produced, when salinity had been reduced to 20 ppt the lipid content was greater than normal salinity of 2.64%, and when salinity was increased to 40 ppt the lipid content produced to be lower, which is 0.19%. Microalgae *Tetraselmis* sp. survive a hypo-osmotic condition (low salinity) is thought to kill the lipid layer is greater when compared to hyper osmotic conditions (high salinity) [16].

Of the three differences in salinity and pH (Table 6) the highest lipid content produced at a more alkaline pH is 9.5 (of all salinity). The treatment with the highest lipid levels (10 ppt & pH 9.5) when compared with the cell density obtained (Table 3) shows an inconsistent comparison. The highest cell density is found in the treatment of 20 ppt & pH of 9.5 was 1426.19 x 10^4 cells/mL, while the highest lipid level was 10 ppt & pH 9.5 has a cell density of 658.33 x 10^4 cells/mL. It is possible for *Tetraselmis* sp. microalgae cells under culture conditions with 10 ppt salinity & pH 9.5 is more adaptable by accumulating more lipids. As revealed by Schenk *et al*.,[26] microalgae types of *Spirulina platensis* choose to maintain their survival by producing more lipids when compared to multiplying cells.

Based on the testing of biosolar samples using a UV-Vis Spectrophotometer and with the same wavelength of 680 nm, an absorbance value of 0.084 was obtained (Table 4). Shown in Table 4, the absorbance values resulting from the biodiesel approached the absorbance value on microalgae *Tetraselmis* sp. salinity treatment of 20 ppt with pH 8 (0.085) and in microalgae *Nitzchia* sp. with salinity treatment in 20 ppt with pH 10 (0.083). So that it can be possible that lipids produced by the microalgae *Tetraselmis* sp., this is pure fatty acid (lipid). Generally lipids produced by microalgae are fatty acids and commonly found are palmitic acid. Palmitic acid is a lipid that has the potential as biodiesel. Palmitic acid also includes fatty acids found in coconut and oil palm plants. Fatty acid content in the microalgae species *Tetraselmis* sp. which has been reviewed by Amini [27], found the type of fatty acid in the form of palmitic acid in the type of microalgae *Tetraselmis* sp. that is equal to 6.82%.

4. Conclusion

The highest population growth from microalgae *Nitzschia* sp. found in the salinity treatment of 20 ppt with 10 pH, while *Porphyridium* sp. in the treatment of salinity 40 ppt with pH 5, and *Tetraselmis* sp. found in a salinity treatment of 20 ppt with a pH of 9.5. The highest lipid content of microalgae *Nitzschia* sp. and *Porphyridium* sp. are in salinity treatment of 40 ppt and pH 5, while the microalgae *Tetraselmis* sp. has the highest lipid level at 10 ppt salinity treatment with pH 9.5.

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