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Lipid Contain of Three Microalgaes 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.

Keywords: *Nitzschia* sp., *Porphyridium* sp., *Tetraselmis* sp., lipid, salinity, 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 of Indonesia waters, is possible to be used for the development of industrial use of microalgae [2]. Microalgae have an important role as a primary producer such as in a food chain, because it has the ability to photosynthesize as a high-level plant by absorbing sunlight, water, and carbon dioxide which then 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 have fairly high lipid content [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 carry out photosynthesis using carbon dioxide (CO_2) and its accumulation results are formed as aside of carbohydrates and lipids. Based on these explanations, it is necessary to study 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 Location 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 Methods

The study was conducted on a laboratory scale with an experiment method. The method that used in this study was Completely Randomized Design (CRD) with factorial 2x2 of mikroalga *Nitzschia* sp. and *Porphyridium* sp., within salinity of 20 and 40 ppt and water pH of 5 and 10. Each of microalgae was using 4 treatments with 3 repetitions. The factorial of *Tetraselmis* sp. 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 was done in a 3 liter glass culture bottle. Microalgae that contained in bottles were given 28 watts or 8.400 lux light bulbs as many as five pieces (see Figure 1). Each bottle is bubbling with an aerator to help the growth of microalgae.
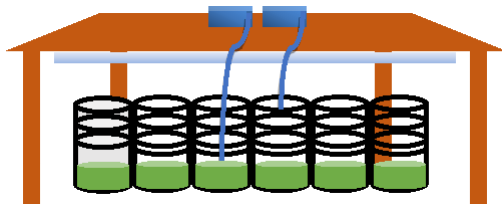

Figure 1 . Illustration of Study Design

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 of Lampung Agency of Mariculture. Then the sea water treated with different pH and salinity as those mentioned in previous 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 aquabidest 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 Lampung Agency of Mariculture 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 study was carried out for 7 days until the peak phase was harvested and a total lipid level was tested at the stationary phase.

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

The cell density of microalgae populations was determined every day using haemo-cytometer on a microscope with a hand counter. As much as 1 mL of sample was taken every day using a dropper pipette and then transferred into a culture bottle. Adding 2-3 drops of formaldehyde was used to facilitate the observation of microalgae. As for the cell density formula used was based on Mudjiman [10], and as follows:

$$T = N \times 25 \times 10^4 \ (\Sigma \ cell/mL)$$

Noted :
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 was determined as follows [11] namely:

$$K = \frac{\log(\frac{Nt}{N_0})}{Tt - T_0} \times 3.22$$

Noted :
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]. Those three microalgae: *Nitzschia* sp., *Porphyridium* sp., and *Tetraselmis* sp. were harvested at the stationary phase or on the 8th day. The microalgae were dropped with NaOH 1 g/L for 24 hours and filtered by using satin fabric to collect them in the form of a paste. The extraction was applied to each treated groups in order to determine the lipid content by using methanol and chloroform with a ratio of 1: 1 or (3 mL: 3 mL) and were homogenized 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:

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

Noted

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 the differences in each treatment. LSD was carried out after obtaining the results of variance analysis. Growth rates and total lipid levels were analyzed descriptively. Microalgae density data were analyzed using a transformation formula according to Fowler *et al.,* [13].

$$= \frac{Tn - T0}{T0} \times 100\%$$

Noted:
T0 : 0-day initial cell cell culture
Tn : n^{th} 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.

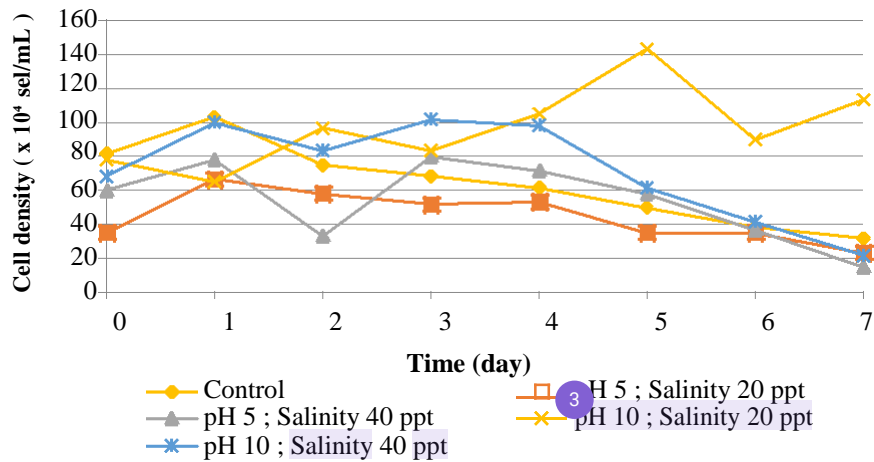The following data indicated density of *Nitzchia* sp. cells on differences in salinity and pH treatment for 7 days.

Figure 2. Cell density of *Nitzschia* sp.

Data on density of *Nitzschia* sp. cell (Figure 2) was highest in pH 10 and salinity of 20 ppt was 143.33 x 10 ^{4} cells/mL on the fifth day, while the lowest sample was on the seventh day of the pH 5 and salinity 40 was 15 x 10 ^{4} sel/mL. On day 5, the number of cell density increased as much 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 decreased to 90 x 10 ^{4} cells/mL. The increase in cell density could be influenced by the nutritional content contained in the media and environmental factors [14]. High nutrient content will provide all nutrients needed by microalgae.

On day 1, the treatment group with pH of 5 and salinity of 20 ppt was different from other treated

groups with the average population growth was 84.72%, which was the highest among others. Yet at the day 4, the treatment group of pH 5 and salinity of 40 ppt reached to the top of population growth. Kawaroe *et al.* [3] indicated that on the 4th to 5th day of cultivation, microalgae were in the exponential final phase and entered the stationary phase. The result of this study (Table 1) also showed similar action that on 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.

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]

Noted: Different letter notations in the same row indicated significant differences

The following are data on the density of *Porphyridium* sp. on differences in salinity and   pH cultured for 7 days.
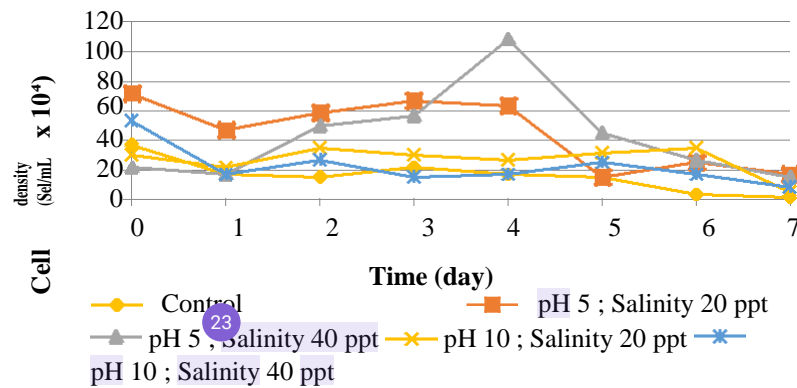
Figure 3. Cell density of *Porphyridium* sp.

The highest *Porphyridium* sp. cell density reached by those in pH of 5 and salinity of 40 ppt (Figure 3). It reached up to 108.33 x 10 [4] cells/mL on the fifth day, while the lowest group was on the sixth day of the control group of 1.67 x 10 [4] cells mL. Based this figure, the sample cell density began to increase on day 4 to 5, and then decreased at the 6th and 7th of culture days. Allegedly on the 4th and 5th day, cells were in the exponential phase, namely cell growth had increased because cells were able to adapt well. When experiencing an exponential phase, microalgae cells were actively reproducing by cleavage [15]. On the 6th and 7th day the cell experienced a decrease because on that day, the cells were 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 pp t	pH 5 & Salinity 20 ppt	pH 5 & Salinity 4 0 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

Noted: 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 was the density of microalgae *Tetraselmis* sp. transformation data from the increase or decrease in the growth population of the treatment groups.

Table 3. *Tetraselmis* sp. Growth population 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

Noted: The different superscript letters indicates difference value between treatments in the BNT test with a significance level 5%
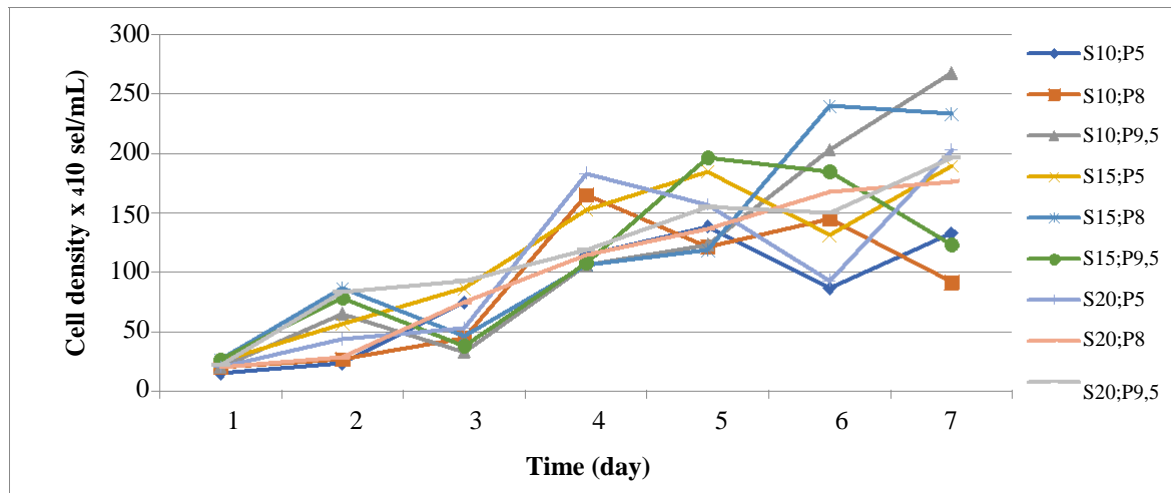
Figure 4 . Cell density of *Tetraselmis* sp. at different salinity and pH

The percentage of addition or decrease from *Tetraselmis* sp. microalgae from highest salinity treatment at 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 at 10 ppt & pH 5 was 200.33% with cell density 133.33 x 10 ^{4} cells/mL (Figure 4). Other study indicated that *Tetraselmis* sp. cells was more tolerant at the condition from the salinity of 20 ppt. Other study indicated that microalgae *Tetraselmis* sp. had the highest percentage of increasing in population at salinity of 20 ppt with cell density of 677.78 x 10 ^{4} cells/mL [16] . Most of the microalgae tolerated the conditions of environmental changes such as salinity with a very large range and most species of microalgae well performed growth in salinity which was 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. had the highest cell density at alkaline pH (pH 9.5), presumably pH 9.5 was suitable for absorption of nutrients by microalgae *Tetraselmis* sp. and the continuation of optimum enzyme activity so that the metabolic process took place quickly and caused cell density to increase. The pH level of the media can affect the workings of enzymes in the process of microalgae cell metabolism [18]. The pH level of media also determine the level from solubility and availability of mineral ions that affect microalgae cells in absorbing nutrients [19]. While the lowest density at pH 5, the cell did not get nutrients properly because of the low pH that interferes with the solubility of mineral ions. The treatment with the initial pH of medium 5, the lowest cell density caused by acidic initial pH can disrupt cell metabolism, and resulting in cells did not optimally absorbing nutrients so that further growth would be disrupted [20].

3.2 Specific population growth of microalgae *Nitzschia* sp., *Porphyridium* sp., and *Tetraselmis* sp.
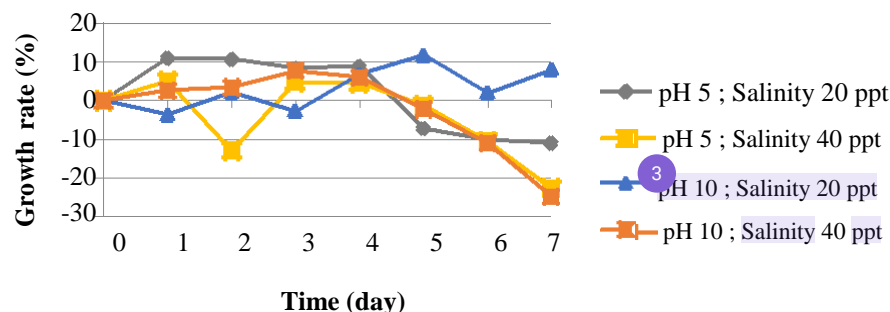
The following graph of the growth rate of *Nitzschia* sp.

Figure 5. Growth rate of *Nitzschia* sp.

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

The higher differences salinity with their original habitat, the more severe for adaptation process in turn the reproduction process and growth was 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 the cell [18].

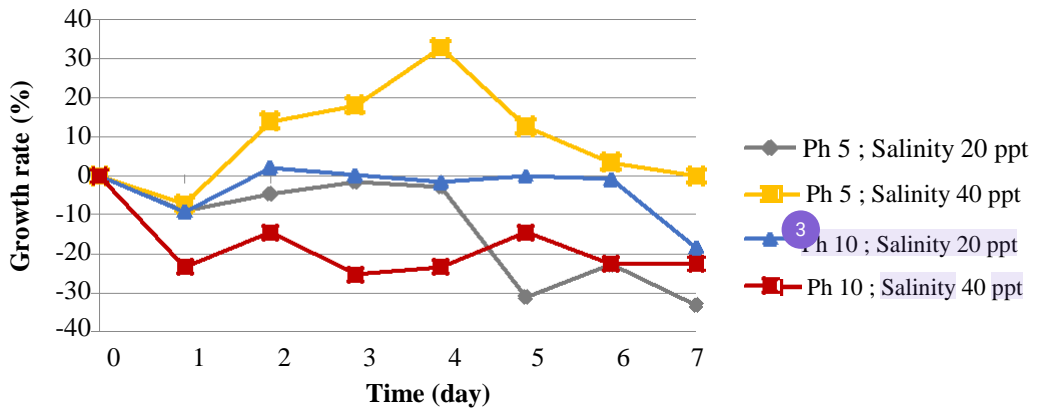The following graph from the growth rate of *Porphyridium* sp.

Figure 6. Growth rate of *Porphyridium* sp.

From this figure (Figure 6), the highest percentage value was 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 then increased sharply on days 3, 4, 5, and 6. This was indicated by a change in the graph line that looks different from the

other treatment samples. This could be due to the degradation of chlorophyll-a [23] which affects fixation of CO_2 becoming decreased and caused the decrease pH value.

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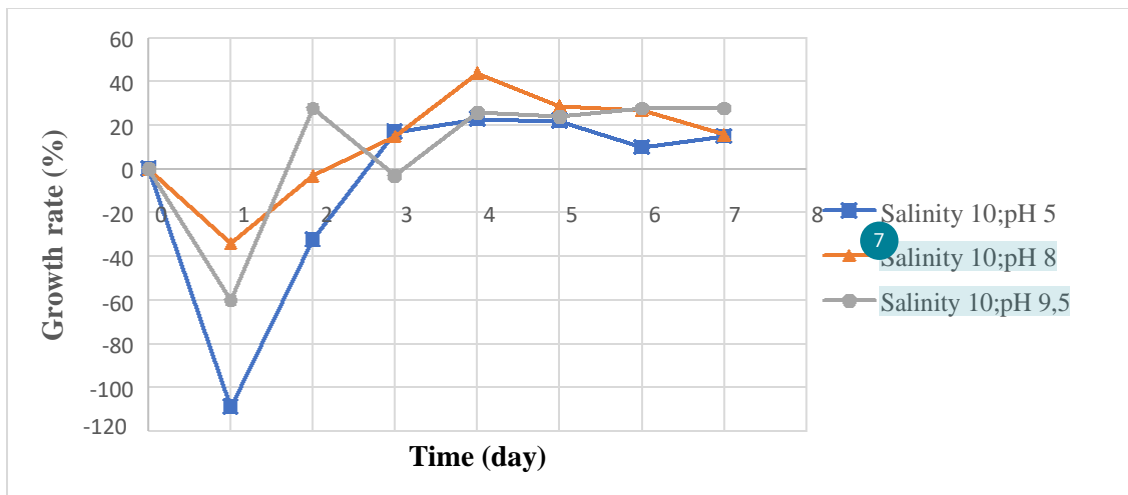

Figure 7. Specific population growth rate of *Tetraselmis* sp. salinity 10 ppt (pH 5; 8; and 9.5)

In Figure 7 indicates that the highest specific growth rate was found on the fourth day at 23%, while at salinity 10

ppt & pH 8 at 44%, and at salinity 10 ppt & pH 9.5 was found on the second, sixth and seventh days at 28%.
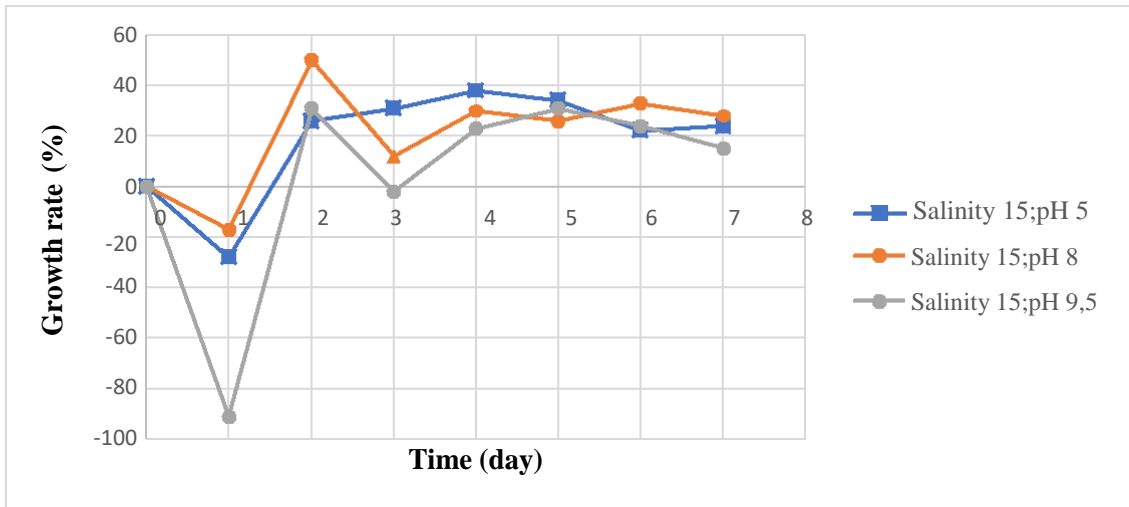
Figure 8. Specific population growth rate of *Tetraselmis* sp. at 15 ppt salinity (pH 5; 8; and 9.5)
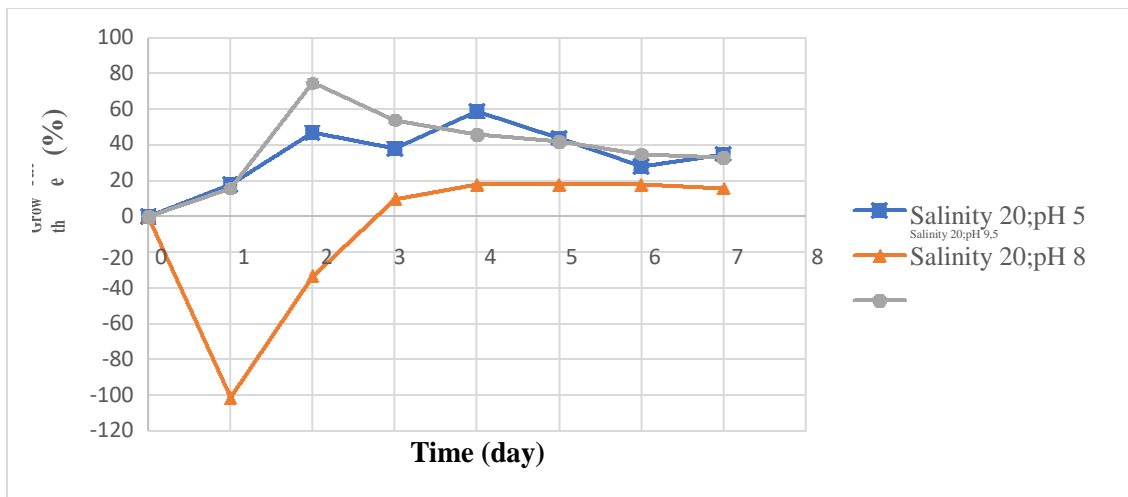

Figure 9. Specific population growth rate of *Tetraselmis* sp. at salinity 20 ppt (pH 5; 8; and 9.5)

In figure 8, for 10 ppt salinity & pH 5 the highest specific growth rate was 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 was found on the second and fifth days at 31%.

In figure 9, the highest specific growth rate for salinity is 20 ppt & pH 5 was on the fourth day 59%, for salinity 20 ppt & pH 8 was 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). This occurrence presumably that cells were still in condition in where treatment media had abundant nutrients, so that microalgae performed in good growth. On the second and fourth day, *Tetraselmis* sp. cells started to enter the exponential phase which was characterized by increasing population growth rates. Ru'yatin *et al*. [24] stated 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. was in stationary phase or static phase, which was 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 occured due to
lack of nutrient supply, therefore their ability to grow was very low [25]. This was 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 10. The results of the three centrifugations were generated, namely the lipid phase, natant, and supernatant. The lipid phase that was used to determine the lipid weight of each sample.

The total lipid levels of microalgae *Nitzschia* sp. , *Porphyridium* sp. , and *Tetraselmis* sp. can be seen in Table 4.
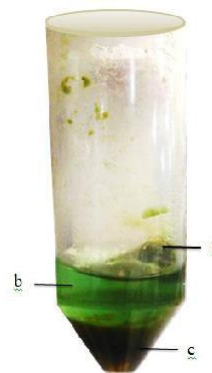
Figure 10. Microalgae pellet after centrifugation (a) natan phase, (b) lipid phase and (c) supernatant phase.

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 Lipid s (%)	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 at pH 4 and salinity 40 ppt of 1.52%, while the lowest lipid was 0.83% in the treatment sample at 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 was 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 were different. The absorbance value of the sample pH 5 and salinity 20 ppt was 0.057 (highest), while the control sample was 0.042.

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, 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%.

In Table 4, *Tetraselmis* sp. microalgae salinity of 10 ppt, 15 ppt, and 20 ppt could possibly produce high lipid levels. This was due to that microalgae *Tetraselmis* sp. resistant to high salinity. Study conducted by Ningsih *et al.* [16], indicated that *Tetraselmis* sp. when given salinity of 30 ppt (normal) lipid level of 1.26% was produced, but when salinity had been reduced to 20 ppt the lipid content was greater than normal salinity reached to 2.64%, and when salinity was increased to 40 ppt the lipid content produced was lower up to 0.19%.

From three differences in salinity and pH (Table 4) the highest lipid content produced at more alkaline pH was 9.5 (of all salinity). The treatment with the highest lipid levels (10 ppt & pH 9.5) when compared with the cell density obtained (see Table 3) showed inconsistent comparison. The highest cell density 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 had a cell density of 658.33 x 10^4 cells/mL. It was possible for *Tetraselmis* sp. cells under culture conditions with 10 ppt salinity & pH 9.5. They were 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. in salinity treatment of 20 ppt with pH 8 (0.085) and in microalgae *Nitzchia* sp. within salinity treatment of 20 ppt with pH 10 (0.083).

4. CONCLUSION

The highest population growth from microalgae *Nitzschia* sp. found in the salinity treatment of 20 ppt with 10 pH, while *Porphyridium* sp. was 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 micro-algae *Nitzschia* sp. and *Porphyridium* sp. were in salinity treatment of 40 ppt and pH 5, while the

microalgae *Tetraselmis* sp. had the highest lipid level at 10 ppt salinity treatment with pH 9.5.

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