Journal of Aquaculture and Fish Health Vol. 11(2) - June 2022
DOI : 10.20473/jafh.v11i2.30388

Growth and Lipid Profiles of *Melosira* sp. in Response to Different Salinity Levels

Indyaswan Tegar Suryaningtyas[1*], Sandi Permadi[2], Solikin[3], Jasmadi[1], Sherly Sapulete[2], Suparmo[2] and Dwi Sunu Widyartini[4]

[1]Research Center for Food Technology and Processing, National Research and Innovation Agency (PRTPP BRIN), Jln. Yogya - Wonosari KM 31,5 Gading, Playen, Gunungkidul, Daerah Istimewa Yogyakarta 55861, Indonesia

[2]Research Center for Oceanography, National Research and Innovation Agency (PRO BRIN), Jl. Pasir Putih 1, Ancol Timur, Jakarta 14430, Indonesia

[3]Research Center for Marine and Fisheries Product Processing and Biotechnology, Jl. K.S. Tubun Petamburan VI, Jakarta, 10260 Indonesia

[4]Faculty of Biology, Universitas Jenderal Soedirman. Jl. Dr. Soeparno 63, Purwokerto, Banyumas 53122, Central Java, Indonesia

*Correspondence :
indyaswantegar@gmail.com

Received : 2021-09-30
Accepted : 2022-05-09

Keywords :
Biomass production, Diatom, Lipid, Melosira, Salinity

Abstract

The Diatom of *Melosira* sp. is one of the potential microalgae candidates for future biofuel resources due to its high lipid profile and fatty acid content, high growth rate, and a quick deposition rate. Salinity stress is one of the environmental factors affecting the growth of microalgae and their lipid content. This research was conducted to identify the response of *Melosira* sp. treated with different salinity levels on the cell growth rate, biomass productivity, and lipid production. The microalgae cells were incubated for 8 days in 1 liter of F/2 growth medium with different salinity levels from 10 ppt, 15 ppt, 20 ppt, 25 ppt, 30 ppt, and 35 ppt. Cell's growth, SGR, biomass, lipid, and water quality factors were measured during incubation. This study revealed that the growth pattern of *Melosira* sp. cells under various salinities was customarily similar. Observation in the 35 ppt salt presenting the biomass productivity was 711.04 ± 69.38 mg/L with lipid productivity which was 60.49 ± 1.72 mg/L. On the other hand, the observation of the lowest salt concentration displaying the biomass productivity was 316.64 ± 16.66 mg/L with the lipid productivity which was 41.46 ± 6.94 mg/L. Hence, the results demonstrated that the lower salinity stress in 10 ppt enabled significant cell's lipid production than the higher salinity of *Melosira* sp. in F/2 medium. Furthermore, lipid productivity was uncorrelated with biomass production patterns. This information may be useful in optimizing *Melosira* sp. lipid performance as a supporting knowledge.

INTRODUCTION

The increase of human population and industrial growth caused the increased demand for fuel, so that alternative fuel and energy is urgently required. Meanwhile, fossil fuel sources are depleting. Hence, it is critical to produce, expand, and utilize biomass derived from non-fossil fuels as a source of

renewable energy raw materials (Suharto, 2017). Microalgae are considered as a potential source of biofuel for the future (Collet *et al*., 2014). The production of microalgae-based biodiesel is promising because it possesses several advantages such as short and sustainable harvest durations throughout the year and superior lipid productivity. Lipid content of microalgae ranges from 20-50% (Selvan *et al*., 2013) and possesses the potential to synthesize lipids 30 times more per hectare than terrestrial plants without competing with agricultural land for food (Graham *et al*., 2012). Therefore, lipid content is a prior characteristic in the study of microalgae species for biodiesel production, and many species have been examined for the growth characteristics and lipid content (Mofijur *et al*., 2019).

The diatom of *Melosira* sp. is one of the potential candidates for biodiesel raw material. *Melosira moniliformis* has a profile of high lipid and fatty acid content (Vilchis *et al*., 2018). Moreover, *Aulacoseira granulata* (*Melosira granulata*) has a high profile of growth rate and lipid content, and also a rapid deposition rate enhancing the harvesting process (Efe *et al*., 2018). *Melosira* sp. is not a food commodity. Thus, it does not compete with food consumption and fulfills one of the criteria as a raw material for biodiesel. *Melosira* is discovered in estuarine waters which possess various salinity ranges (Van de Vijver and Crawford, 2020; Yuan *et al*., 2020). Potapova (2011) reported that *Melosira* sp. was identified in waters with a salinity range of 0.5-30 ppt.

Environmental conditions and microalgae growing media can affect lipid content (Pandit *et al*., 2017). It is further explained that these environmental factors include nutrition, temperature, light intensity, lighting duration, salinity level, and nitrogen content in the growing medium (Vilchis *et al*., 2018; Rukminasari *et al*., 2021). In optimum condition, microalgae perform a high-rate biomass productivity, but relatively with a low lipid content. Conversely, most microalgae with higher lipid content possess a slower growth rate. Therefore, low salinity stress is considered as one of effective strategies to enhance lipid content in microalgae cells (Church *et al*., 2017; Atikij *et al*., 2019).

This study was performed to evaluate the response of *Melosira* sp. isolates which were treated with different salinity levels on cell growth rate, biomass productivity and lipid production. Hence, they become the basis for biodiesel feedstock development on a large scale.

METHODOLOGY

Place and Time

In controlled laboratory conditions (Research Center for Oceanography, Indonesian Institute of Sciences in Jakarta, August-October 2018) this study of *Melosira* sp. were established.

Research Materials

The instruments used in this study were hemocytometer (Improved Neubauer-Germany), hand counter, binocular microscope (Nikon-Japan), Metrohm 780 pH Meter (Switzerland), Milwaukee SM700 portable luxmeter (Hungary), ATAGO hand refractometer S/Mill (Japan), oven (Memmert-Germany), centrifuge, vortex, Duran bottle-Germany, analytic balance, vacuum pump, and autoclave. The materials included were *Melosira* sp., F/2 medium ($NaNO_3$, NaH_2PO_4, $NaSiO_3.9H_2O$, $FeCl_3.6H_2O$, $Na_2EDTA.2H_2O$, $MnCl_2.4H_2O$, $ZnSO_4.7H_2O$, $CoCl_2.6H_2O$, $CuSO_4.5H_2O$, $Na_2MoO_4.2H_2O$ (Merck), Thiamine, HCl (vitamin B1), Biotin, vitamin B12), filter paper (GF/C Glass Circles Whatman).

Research Design

Three repetitions of enriched seawater containing six different salt concentrations (10 ppt, 15 ppt, 20 ppt, 25 ppt, 30 ppt, and 35 ppt) were administered to determine the effect of varying salinity levels on the growth performance, biomass, and lipids of *Melosira* sp. Cell growth was counted daily (8 days), meanwhile water parameters

and light were recorded in the beginning and the end. At the end of the incubation, biomass and total lipid were determined.

Work Procedure

Microorganism and Cultivation Condition

Melosira sp. obtained from RCO, Indonesian Institute of Sciences in Jakarta acclimated as a single culture. All apparatus were in sterile condition, autoclaved at temperature 121°C, 2 atm pressure for ±15 minutes then 45 °C in an oven for 12 hours. *Melosira* sp. was cultivated in 25 ppt seawater enriching with f/2 medium. f/2 medium nutrient was formulated from Andersen (2005). It consists of macronutrients (8,82.10^{-4} M $NaNO_3$, 3,62.10^{-5} M NaH_2PO_4, and 1,06.10^{-4} M $NaSiO_3.9H_2O$), a trace metal solution (comprising of 1,17.10^{-5} M $FeCl_3$.6H2O, 1,17.10^{-5} $Na_2EDTA.2H_2O$, 9,10.10^{-7} M $MnCl_2.4H_2O$, 7,65.10^{-8} M $ZnSO_4.7H_2O$, 4,20.10^{-8} M $CoCl_2.6H_2O$, 3,93.10^{-8} M $CuSO_4.5H_2O$, dan 2,6.10^{-8} M $Na_2MoO_4.2H_2O$), and vitamin solutions (consisting of 2,96.10^{-7} M Thiamine, HCl (vitamin B1), 2,05.10^{-9} M Biotin (vitamin H), and 3,96.10^{-10} M (Cyanocobalamin or vitamin B12). 1 ml nutrient stock solution f/2 was applied to 1 L cultivation volume. Seawater media was filtered with screen (40 μm) then autoclaved on temperature 121 °C, 1 atm for ±15 minutes.

Different salinity seawater was provided by mean of applying distilled water for salinity adjustment. Three replicate sterile culture media were formulated for some different salinity, which were 10 ppt, 15 ppt, 20 ppt, 25 ppt, 30 ppt, and 35 ppt. The culture condition was modified from Vilchis *et al*. (2018)'s approach. Approximately, 50.000 cells/ml were cultivated in each different salinity medium previously prepared. These were continuously aerated and illuminated 12 hours/day on white illumination (± 1500 lux) until the end of incubation. Aeration was provided for homogenizing the nutrient, avoiding clumping, and distributing the cells to obtain optimum light for cells photosynthesis. Cells were incubated for 8 days under temperature 25-30 °C.

Cells Growth and Water Quality Factors Measurement

Cells growth was calculated daily using hemocytometer and hand counter under binocular microscope (Isnansetyo and Kurniastuty, 1995). By utilizing sterile glass Pasteur pipette (treated by alcohol), approximately, 2 ml *Melosira* sp. cultivation was collected to a new tube for each cultivation. Promptly after cell collection, it was determined utilizing hand counter and *haemocytometer improved* Neubauer under light microscope (Nikon Optiphot-2) on proper magnification. Cells density was estimated with following formula:

$$CD = \frac{50.000 \times (CC\ 1 + CC\ 2)}{2}$$

CD = cells density (cells/ml)
CC 1 = counted cells chamber 1
CC 2 = counted cells chamber 2

Medium pH was detected by employing pH Meter. Its probe was submersed into *Melosira* sp. cultivation, stable number presented by pH meter digital screen as amount of pH. Light intensity was calculated by Luxmeter. In obtaining light intensity, the lux meter's sensor was directed to the light source (40-watt white fluorescent tube lamp). Then, salinity was measured by utilizing the Hand Refractometer. At the end of incubation, 30 ml of culture was filtered with filter paper (glass microfiber filter 37mm) dried at 45 °C overnight (filter weighed as W_0) in an oven previously. Filtering was administered with a vacuum pump to obtain excellent yield when filtering. Subsequently, filtered cells on the filter paper were dried at 45 °C overnight in the oven (weighed as W_t). Cell's biomass was collected by W_t-W_0 then converted to a unit gram/L, biomass productivity was the biomass divided per day.

Growth rate analysis was elaborated by following equation (Wood *et al*., 2005):

$$SGR = \frac{Ln \frac{N_t}{N_0}}{\Delta t}$$

SGR = specific growth rate (μ/day)
N_t = the cells peak growth (cells/ml
N_0 = the initial cells density (cells/ml)
Δt = length of time interval

Lipid Determination

Lipids were determined after extraction by Bligh and Dyer (1959) with slight modification. Two kinds of solvents were implemented, chloroform: ethanol (1:2 v/v = solution A). The phytoplankton biomass and solution A were homogenized on the vortex by several minutes. Then, distilled water was applied in the same proportion as chloroform (ratio 1:1 v/v) to the suspension, then it was homogenized on the vortex for ±30 seconds. The suspension was centrifugated at 3000 rpm for 15 minutes, 25 °C. The upper layer was transferred to the new tube, then methanol: chloroform (1:2 v/v) solution was administered to the rest for 15 minutes sonication process.

The generated additional upper layer was then transferred to the prior tube. The bottom phase was delivered to a dish (with the empty portion pre-weighed) and evaporated at an oven temperature of 80 °C. After cooling the dish in a desiccator, it was weighed. Then, according to Kim *et al*. (2016), the lipid total content extracted from the cells was evinced as the weight % of dried cell weight. Lipid yield was attained by lipid weight per one liter cell culture and the lipid productivity was lipid yield per day culture.

Data Analysis

The analysis data were statistically evaluated administering one-way ANOVA (Minitab.16) to determine differences in mean values. The significance level was installed at 95%.

RESULTS AND DISCUSSION

Melosira sp. is a species considered as a lipid source, in which it is able to produce a high amount of lipid and fatty acid (Vilchis *et al*., 2018). In examining the influence of salinity on the growth response and lipid production of *Melosira* sp., cell growth, biomass, and lipid content, they were observed under a wide range of salinity (10-35 ppt).

Cells Growth

Generally, cell growth of the *Melosira* sp. under different salinities displayed the same pattern on day 1 to 8 (Figure 1). Our results demonstrated that *Melosira* sp. was relatively tolerant to salinities between 10 ppt and 35 ppt although there were slightly different growth patterns. Similarly, prior study has revealed that *Melosira* sp. was discovered in estuaries with a wide range of salinity (0.5-30 g/L) (Khelfeh and Esmaeili, 2009; Potapova, 2011; Pratiwi *et al*., 2018). Ordinarily, lag, exponential, stagnant, and death phases occurred during *Melosira* sp.'s life cycle in this study, it is a prevalent cycle in microalga growth (Lavens and Sorgeloos, 1996).

This finding indicated that the lag phase occurred two days prior to day 3, but the salinity of 30 ppt and 35 ppt in high salinity culminated until day 5. The longer lag phase could also be associated with the medium condition, as it occurred with *M. moniliformis* (Vilchis *et al*., 2018). It is recognized that lag phase occurs as a result of physiological adaptation of the cell metabolism. Furthermore, various explanations for the lag stage in newly transplanted cultures encompass the cell population being in a degraded state, the cells undergoing initial adaptation to the culture medium, and the presence of non-viable cells (Lavens and Sorgeloos, 1996, Vonshak, 1985). After the lag phase, the cells grew and acquired their maximum density before encountering the dying phase. The highest number of cells in 10 ppt's peak growth reached 1,775,000 cells/ml, followed by 30 ppt and 35 ppt, totaling 1,641,667 cells/ml and 1,583,333 cells/ml correspondingly.

On the other hand, the Specific Growth Rate of *Melosira* sp. decreased

with increasing salinity of the growth medium (Table 1). Lower salinities were determined in a comparatively greater SGR, with the highest being at 15 ppt, which was merely marginally different from 10 ppt that was 0.83 ± 0.041 μ/day and 0.71 ± 0.049 μ/day respectively ($p < 0.05$). Meanwhile, the SGR of 35 ppt was the lowest that was 0.45 ± 0.032 μ/day. The SGR's descent was associated with the culture's peak day, with the longer the peak day, the lower the SGR in this case study. In the higher medium salinities, the microalgae required a longer period to adapt and grow, such as *Melosira moniliformis* (Woelfel *et al*., 2014). In our experiment, it demonstrates that peak day is related to the cultivation's efficiency. To a cultivator, a shorter peak day of cultivation is frequently preferable to a longer one, as it may curtail production costs and occasionally be more efficient in mass production over a short period of time.

Table 1. Growth rate (mean ± SD), peak of growth (day) and (cells/ml) of *Melosira* sp., cultivated at various salinity levels.

Salinity (ppt)	SGR (/day)	Peak (day)	Peak (cells/ml)
10	0.71 ± 0.049^{b}	6	1,775,000
15	0.83 ± 0.041^{a}	5	1,400,000
20	0.63 ± 0.015^{bc}	6	1,191,667
25	0.58 ± 0.046^{c}	7	1,641,667
30	0.57 ± 0.051^{c}	7	1,583,333
35	0.45 ± 0.032^{d}	8	1,166,667

Note: different letters indicate significant differences between means ($n = 3$, $p < 0.05$).

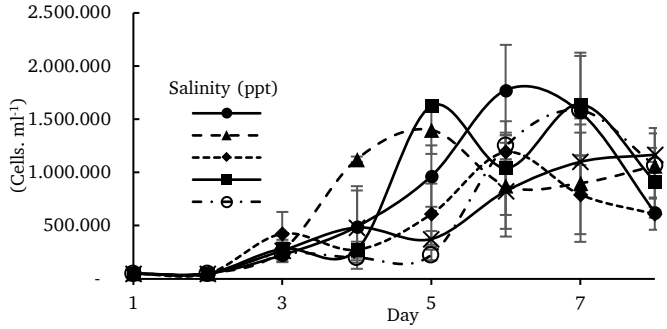

Figure 1. Means of cell density ± SD of *Melosira* sp., cultivated at various salinity levels.

Biomass

Unlike peak day and SGR of microalgae cultivation, biomass refers to the actual mass of cells attained during a single cultivation period. Biomass is employed to determine the efficiency of microalgae cultivation as an end-product. The salinity culture medium treatments influenced the biomass of *Melosira* sp. Statistically ($p < 0.05$), the biomass presented a range of responses, with the lower the salinity of the medium, the less biomass was obtained from the culture. The biomass was acquired at the end of growth at the lowest salinity of 10 ppt which was 316.64 ± 16.66 mg/L. The biomass increased gradually as the medium salinity in which they were grown increased. In the most salted medium (35 ppt), on the other hand, biomass appeared to reach 711.04 ± 69.38 mg/L but it is lower than *Melosira* sp. studied by Prayitno *et al*. (2020), it was caused by the higher initial density and the longer incubation day.

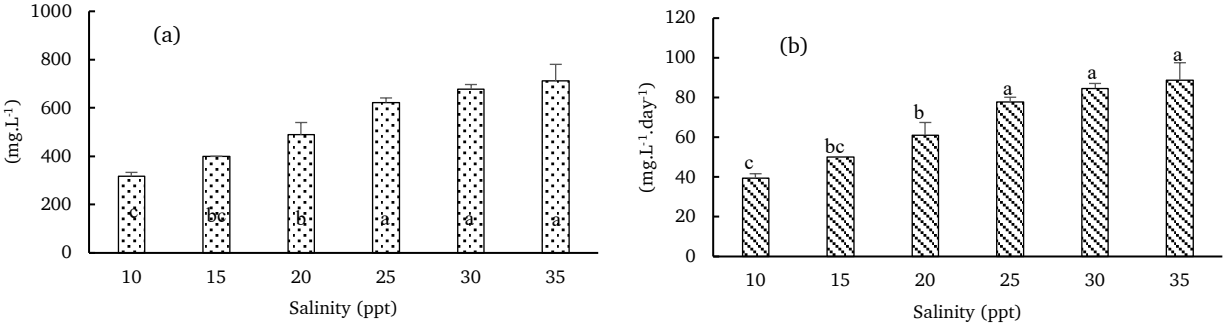

Figure 2. Means of biomass (a) and biomass productivity (b) under different salinity medium. Vertical bars are means ± SD (n = 3). Means with different letters are significantly different at $p<0.05$.

Statistically, the cell biomass of the two lowest salinities were significantly different ($p<0.05$) with the three largest medium salinities (25 ppt, 30 ppt, and 35 ppt). The greater salinities (>20 ppt) in this study provided favorable conditions for *Melosira* sp. biomass production. Moreover, harvesting the culture on its peak growth day may contribute to the higher biomass collected. According to Anwar *et al*. (2018), the higher dried biomass was attained concurrently with an apparent increase in the density of culmination cells. Then, some patents also indicate to take advantage of this condition by harvesting at the stage, during the peak phase (Tabernero *et al*., 2013).

Similarly, the biomass productivity of *Melosira* sp. (milligram per liter per day) followed the same trend as the biomass, because biomass productivity was determined by the amount of biomass grown per day. The highest yield was 88.88 ± 8.67 mg/L/day in the medium salinity of 35 ppt. On the contrary, the least was 39.58 ± 2.08 mg/L/day at the lowest medium salinity. According to cell biomass and productivity data, the deployable salinity range for obtaining a good yield is at 25 ppt to 35 ppt. Nonetheless, when economic efficiency is considered, it is possible to administer 25 ppt medium salinity in which the yield did not differ much from 35 ppt salinity. However, *Melosira* sp. growth has not been terminated, as demonstrated by the final three medium salinity treatments (Figure 2). In this regard, *Melosira* sp. indicates to be capable of being grown in a higher salinity media for the increased yield, or probably 35 ppt is the maximum salinity for cultivating *Melosira* sp.

Lipid

Lipid accumulation occurs when microalgae are cultivated under specific or extreme conditions, which can be induced artificially. Sun *et al*. (2018) explained that lipids may have a role in cells' resistance to salt stress. Modifying salinity throughout the growth of *Melosira* sp. in this study possessed a significant influence on lipid content (%), as illustrated in figure 3. In contrast to the previous explanation, the lowest salinity (10 ppt) resulted in the most lipid accumulation in the cells, up to 13.25 %. Meanwhile, higher salinity was obtained in the lowest lipid accumulation in the cells (8.58 %) but it is still larger than the lipid of *Melosira moniliformis* (4.44% – 8.30%) examined by Vilchis *et al*. (2018). These findings appeared to be consistent with previous studies by García *et al*. (2012), in which the diatom *Thalassiosira weissflogii* exhibited a similar response to lipid content when the cells were grown at a greater salinity (25 ppt to 50 ppt). However, *Chaetoceros* cf. *wighamii*, another diatom, did not reveal a significant lipid synthesis reaction in

response to the increased medium salinity (Araújo and Garcia, 2005). In this regard, salinity stress is sufficient to induce a variety of cell responses depending on the microalgae species and environmental conditions. In accordance with prior findings, microalgae's response to salinity variation was species-specific, and cell growth also varied between species (Kumar *et al.*, 2020).

Meanwhile, the production of lipid attained at the end of microalgal growth presented a distinct perspective, with the higher salinities producing more lipid (Table 2). The maximum lipid production was 60.49 ± 1.72 mg/L at the highest salinity (35 ppt), and statistically, significantly different ($p<0.05$) from the lowest salinity, 41.46 ± 6.94 mg/L which was equivalent ($p<0.05$) to 15 ppt and 20 ppt culture. This finding is consistent with the biomass at the end of cultivation, a larger lipid production was obtained by extracting a greater amount of biomass. Corroborating with previous study, the higher cell growth rate resulted in the best lipid productivity in *Dunaliella tertiolecta* (Rizwan *et al*., 2017). Besides, as Lynn *et al*. (2000) confirmed, low salinity may result in decreased cell dimension which likely leads to the end of the smaller microalgae biomass, hence, influences the lipid production.

Furthermore, this finding revealed that the higher biomass collected on 35 ppt medium demonstrated that harvesting earlier during peak growth was allowed for greater biomass (Figure 2). Thus, it is probably possible to harvest *Melosira* sp. at the lowest medium salinity on the peak day in order to obtain significantly more biomass and lipid production. Moreover, according to the findings in Figure 3, the percent lipid content of the low medium salinity was higher, which indicates that this medium is a promising cultivation medium for *Melosira* sp. lipid production. Meanwhile, lipid yield was unveiled to be similar to the number of lipid production, with 15 ppt and 10 ppt were being the lowest at about 5.02 ± 0.11 mg/L/day and 5.18 ± 0.87 mg/L/day respectively and significantly different ($p<0.05$) from 35 ppt medium as the most productive medium (7.56±0.21 mg/L/day) and 25 ppt (7.49±1.14 mg/L/day) (Table 2).

Table 2. *Melosira* sp. lipid production and yield (mean ± SD) grown under various salinity levels.

Salinity	Lipid production ($mg.L^{-1}$)	Lipid yield ($mg.L^{1}day^{-1}$)
10	41.46 ± 6.94^{b}	5.18 ± 0.87^{b}
15	40.19 ± 0.88^{b}	5.02 ± 0.11^{b}
20	42.05 ± 6.61^{b}	5.26 ± 0.83^{b}
25	59.94 ± 9.15^{a}	7.49 ± 1.14^{a}
30	52.75 ± 2.60^{ab}	6.59 ± 0.32^{ab}
35	60.49 ± 1.72^{a}	7.56 ± 0.21^{a}

Note: Different letters indicate significant differences between means (n = 3, p < 0.05).

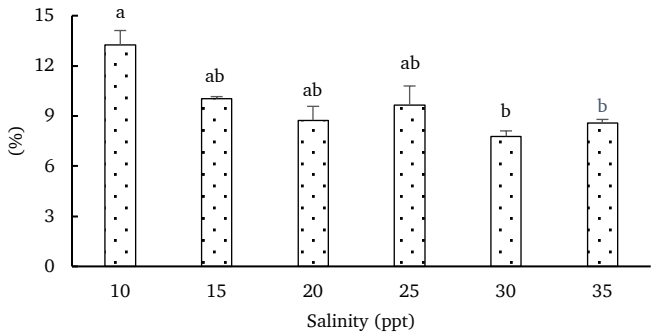

Figure 3. Lipid content of *Melosira* sp. under different salinity levels. Vertical bars are means ± SD (n = 3). Means with different letters are significantly different at $p<0.05$.

Environmental Factors of Cells Growth

The result revealed that there was no significant change in pH. The decrease was predominantly caused by a change in the metabolism of microalgae when they were utilizing medium nutrients for growth. As explained by Xin *et al*. (2010), employing ammonium as a nitrogen source enabled algae to release a proton to maintain cell neutrality, which acidified the media. In addition, bacterial growth also contributes to the declining pH medium (Yang *et al*., 2018).

Table 3. pH (mean ± SD), temperature and light of the cultivation media during cells incubation under different salinity levels.

Salinity	pH		Temperature	Light Intensity (Lux)	
(ppt)	D-0	D-8	(°C)	D-0	D-8
10	7 ± 0.00	6.33 ± 0.58	25-30	1346.33 ± 148.65	1344.67 ± 144.31
15	7 ± 0.00	5.67 ± 0.58	25-30	1311.67 ± 115.58	1312.33 ± 115.70
20	7 ± 0.00	6.33 ± 0.58	25-30	1372.33 ± 153.70	1372.33 ± 154.37
25	7 ± 0.00	6.67 ± 0.58	25-30	1372.00 ± 172.65	1360.33 ± 187.01
30	7 ± 0.00	6.67 ± 0.58	25-30	1363.00 ± 139.75	1361.00 ± 143.15
35	7 ± 0.00	6.33 ± 0.58	25-30	1309.33 ± 94.11	1277.67 ± 146.19

Unlike pH, the temperature increased from 25 °C to 30 °C at the end of growing. It was still within the range for growth, because this microalga has previously been acclimated to this temperature range in a laboratory collection. Furthermore, naturally, *Melosira* sp. is uncovered in Indonesian waters and grows normally at 28-34°C (Indrayani *et al*., 2018). Temperature changes are possibly as a result of the fluorescent bulb delivering heat energy into the microalgae growing medium. Brzychczyk *et al*. (2020) also postulated that temperature changes in the photobioreactor were generated by LED illumination during the incubation process. On the other hand, light was regarded constant in intensity between 1293.50 and 1366.17 lux. Lavens and Sorgeloos (1996) confirmed that the light intensity for microalgae is between 1,000-10,000 lux, and in the laboratory, 1,000 lux enabled optimal growth conditions for another marine diatom like *Chaetoceros muelleri* (Pal *et al.*, 2013).

CONCLUSION

In general, our data demonstrate that various salinity treatments possess a significant effect on the lipid yield of *Melosira* sp. This aspect of the research revealed that the lowest salinity of 10ppt enables the synthesis of large lipid content in comparison to *Melosira* sp. normal growth salinity. However, lipid productivity was obtained at the higher salinity medium due to its high biomass. The finding corroborates that low salinity stress influences *Melosira* sp. cells' lipid production. On the contrary, it decreases the growth rate. This knowledge contributes to a scientifically based strategy for optimizing microalgae lipid synthesis. However, further studies with more focus on the effect of low salinity on lipid production of *Melosira* sp. is therefore suggested.

ACKNOWLEDGMENT

This work was financially supported by the Research Program of the Research Center for Oceanography, Indonesian Institute of Sciences.

REFERENCES

Andersen, R.A., 2005. *Algal culturing techniques,* Burlington, USA, Elsevier Academic Press.

Anwar, S.H., Harzaki, S., Sulaiman, M.I. and Rinanda, T., 2018. Utilization of different nitrogen sources for the growth of microalgae isolated from mangrove leaves in Banda Aceh - Indonesia. *IOP Conference Series: Earth and Environmental Science,*

207, 12049. https://doi.org/10.1088/1755-1315/207/1/012049

Araújo, S.D.C. and Garcia, V.M.T., 2005. Growth and biochemical composition of the diatom *Chaetoceros* cf. *wighamii* brightwell under different temperature, salinity and carbon dioxide levels. I. Protein, carbohydrates and lipids. *Aquaculture, 246*(1-4), pp.405-412. https://doi.org/10.1016/j.aquaculture.2005.02.051

Atikij, T., Syaputri, Y., Iwahashi, H., Praneenararat, T., Sirisattha, S., Kageyama, H. and Waditee-Sirisattha, R., 2019. Enhanced lipid production and molecular dynamics under salinity stress in green microalga *Chlamydomonas reinhardtii* (137C). *Marine drugs, 17*(8), p.484. https://doi.org/10.3390/md17080484

Bligh, E.G. and Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Canadian *Journal of Biochemistry and Physiology, 37*, pp.911-917. https://doi.org/10.1139/o59-099

Brzychczyk, B., Hebda, T. and Pedryc, N., 2020. The influence of artificial lighting systems on the cultivation of algae: The example of *Chlorella vulgaris*. *Energies, 13*(22), 5994. https://doi.org/10.3390/en13225994

Church, J., Hwang, J.H., Kim, K.T., Mclean, R., Oh, Y.K., Nam, B., Joo, J.C. and Lee, W.H., 2017. Effect of salt type and concentration on the growth and lipid content of *Chlorella vulgaris* in synthetic saline wastewater for biofuel production. *Bioresource Technology, 243*, pp.147-153. https://doi.org/10.1016/j.biortech.2017.06.081

Collet, P., Lardon, L., Hélias, A., Bricout, S., Lombaert-Valot, I., Perrier, B., Lépine, O., Steyer, J.P. and Bernard, O., 2014. Biodiesel from microalgae-Life cycle assessment and recommendations for potential improvements. *Renewable Energy, 71*(C), pp.525-533. https://doi.org/10.1016/j.renene.2014.06.009

Efe, Ş., Ceviz, M.A. and Temur, H., 2018. Comparative engine characteristics of biodiesels from hazelnut, corn, soybean, canola and sunflower oils on DI diesel engine. *Renewable Energy, 119*, pp.142-151. https://doi.org/10.1016/j.renene.2017.12.011

García, N., López-Elías, J.A., Miranda, A., Martínez-Porchas, M., Huerta, N. and García, A., 2012. Effect of salinity on growth and chemical composition of the diatom *Thalassiosira weissflogii* at three culture phases. *Latin American Journal of Aquatic Research, 40*(2), pp.435-440. https://doi.org/10.3856/vol40-issue2-fulltext-18

Graham, J.M., Graham, L.E., Zulkifly, S.B., Pfleger, B.F., Hoover, S.W. and Yoshitani, J., 2012. Freshwater diatoms as a source of lipids for biofuels. *Journal of Industrial Microbiology and Biotechnology*, *39*(3), pp.419-428. https://doi.org/10.1007/s10295-011-1041-5

Indrayani, Haslianti and Asriyana, 2018. Isolation and screening of marine microalgae from Kendari waters, Southeast Sulawesi, Indonesia suitable for outdoor mass cultivation in hypersaline media. *AACL Bioflux, 11*(5), pp.1445-1455. http://www.bioflux.com.ro/docs/2018.1445-1455.pdf

Isnansetyo, A. and Kurniastuty 1995. *Teknik kultur phytoplankton dan zooplankton: Pakan alami untuk pembenihan organisme laut.* Kanisius, Yogyakarta.

Khelfheh, N.M. and Esmaeili, F., 2009. Composition and frequency and impact of the salinity on distribution phytopankton in the Bahmanshir tidal river. *Iranian Scientific Fisheries Journal, 18*(3), pp.43-51. https://dx.doi.org/10.22092/isfj.2009.115492

Kim, G., Lee, C.H. and Lee, K., 2016. Enhancement of lipid production in

marine microalga *Tetraselmis* sp. through salinity variation. *Korean Journal of Chemical Engineering, 33*, pp.230-237. https://doi.org/10.1007/s11814-015-0089-8

Kumar, S.D., Sojin, K., Santhanam, P., Dhanalakshmi, B., Latha, S. and Kim, M., 2020. Reciprocal response of nitrogen for enhancing growth and proximate compositions of marine microalga *Tetraselmis* sp. under low saline conditions. *Indian Journal of Geo Marine Science*, *49*(2), pp.26-332. http://nopr.niscair.res.in/bitstream/123456789/54066/3/IJMS%2049%282%29%20326-332.pdf

Lavens, P. and Sorgeloos, P., 1996. *Manual on the production and use of live food for aquaculture*, FAO Fisheries Technical Paper No 361. p.295.

Lynn, S.G., Kilham, S.S., Kreeger, D.A. and Interlandi, S.J., 2000. Effect of nutrient availability on the biochemical and elemental stoichiometry in the freshwater diatom *Stephanodiscus minutulus* (Bacillariophyceae). *Journal of Phycology, 36*(3), pp.510-522. https://doi.org/10.1046/j.1529-8817.2000.98251.x

Mofijur, M., Rasul, M.G., Hassan, N.M.S. and Nabi, M.N., 2019. Recent development in the production of third generation biodiesel from microalgae. *Energy Procedia, 156*, pp.53-58. https://doi.org/10.1016/j.egypro.2018.11.088

Pal, S.W., Singh, N.K. and Azam, K., 2013. Evaluation of relationship between light intensity (lux) and growth of *Chaetoceros muelleri*. *Oceanography*, *1*(3). https://doi.org/10.4172/2332-2632.1000111

Pandit, P.R., Fulekar, M.H. and Karuna, M.S.L., 2017. Effect of salinity stress on growth, lipid productivity, fatty acid composition, and biodiesel properties in *Acutodesmus obliquus* and *Chlorella vulgaris*. *Environmental Science and Pollution Research, 24*, pp.13437-13451. https://doi.org/10.1007/s11356-017-8875-y

Potapova, M., 2011. Patterns of Diatom Distribution In Relation to Salinity. *In:* J., S. and P., K. (eds.) *The Diatom World: Cellular Origin, Life in Extreme Habitats and Astrobiology*. Springer, Dordrecht.

Pratiwi, H., Damar, A. and Sulistiono, 2018. Phytoplankton community structure in the Estuary of Donan River, Cilacap, Central Java, Indonesia. *Biodiversitas, 19*(6), pp.2104-2110. https://doi.org/10.13057/biodiv/d190616

Prayitno, J., Rahmasari, I.I. and Rifai, A., 2020. The effect of harvesting frequency on biomass production of *Chlorella* sp. and *Melosira* sp. for biological carbon capture. *Jurnal Teknologi Lingkungan, 21*(1), pp.23-30. https://doi.org/10.29122/jtl.v21i1.3777

Rizwan, M., Mujtaba, G., Rashid, N. and Lee, K., 2017. Enhancing lipid poduction of *Dunaliella tertiolecta* by manipulating the interactive effect of salinity and nitrogen. *Chemical and Biochemical Engineering Quarterly, 31*(3), pp.199-207. https://doi.org/10.15255/CABEQ.2017.1092

Rukminasari, N., Omar, S.A. and Lukman, M., 2021. Temperature and nitrate concentration effect on the abundance and growth rate of *Melosira* sp. *Biolink Jurnal Biologi Lingkungan Industri Kesehatan, 7*(2), pp.185-194. https://doi.org/10.31289/biolink.v7i2.4054

Selvan, K.B., Revathi, M., Piriya, S.P., Vasan, T.P., Prabhu, I.G.D. and Vennison, J.S., 2013. Biodiesel production from marine cyanobacteria cultured in plate and tubular photobioreactors. *Indian Journal of Experimental Biology, 51*(3), pp.262-268. http://hdl.handle.net/123456789/16129

Suharto, 2017. *Bioteknologi dalam bahan bakar nonfosil*. Andi, Yogyakarta.

Sun, X.M., Ren, L.J., Zhao, Q.Y., Ji, X.J. and Huang, H., 2018. Microalgae for the production of lipid and carotenoids: A review with focus on stress regulation and adaptation. *Biotechnology for Biofuels and Bioproducts, 11*, 272. https://doi.org/10.1186/s13068-018-1275-9

Tabernero, A., del Valle, E.M.M. and Galan, M.A., 2013. Microalgae technology: A patent survey. *International Journal of Chemical Reactor Engineering, 11*, pp.733-763. https://doi.org/10.1515/ijcre-2012-0043

Van De Vijver, B. and Crawford, R.M., 2020. *Melosira jeanbertrandiana*, a new *Melosira* species (Bacillariophyceae) from the sub-Antarctic region. *Botany Letters, 167*(1), pp.50-56. https://doi.org/10.1080/23818107.2019.1688677

Vilchis, M.C.L., Pilco, J.I.H., Fuerte, F.O.L. and Rojas, C.A.P., 2018. Growth rate, lipid, fatty acids, and pigments content of *Melosira moniliformis* (Bacillariophyta) in laboratory cultures. *Revista Latinoamericana de Biotecnología Ambiental y Algal, 9*, pp.1-16. http://www.solabiaa.org/ojs3/index.php/RELBAA/article/view/60

Vonshak, A., 1985. Micro-algae: Laboratory growth techniques and outdoor biomass production. *In:* Coombs, J., Hall, D. O., Long, S. P. and Scurlock, J. M. O. (eds.) *Techniques in Bioproductivity and Photosynthesis (Second Edition)*. Pergamon.

Woelfel, J., Schoknecht, A., Schaub, I., Enke, N., Schumann, R. and Karsten, U., 2014. Growth and photosynthesis characteristics of three benthic diatoms from the brackish southern Baltic Sea in relation to varying environmental conditions. *Phycologia, 53*(6), pp.639-651. https://doi.org/10.2216/14-019.1

Wood, A.M., Everroad, R.C. and Wingard, L.M., 2005. *Measuring growth rates in microalgal cultures,* Elsevier Academic Press, Burlington.

Xin, L., Hu, H.Y., Ke, G. and Jia, Y., 2010. Growth and nutrient removal properties of a freshwater microalga *Scenedesmus* sp. LX1 under different kinds of nitrogen sources. *Ecological Engineering, 36*(4), pp.379-381. https://doi.org/10.1016/j.ecoleng.2009.11.003

Yang, E., Fan, L., Yan, J., Jiang, Y., Doucette, C., Fillmore, S. and Walker, B., 2018. Influence of culture media, pH and temperature on growth and bacteriocin production of bacteriocinogenic lactic acid bacteria. *AMB Express, 8*, 10. https://doi.org/10.1186/s13568-018-0536-0

Yuan, M., Jiang, C., Weng, X. and Zhang, M., 2020. Influence of salinity gradient changes on phytoplankton growth caused by sluice construction in Yongjiang river estuary area. *Water, 12*(9), 2492. https://doi.org/10.3390/w12092492