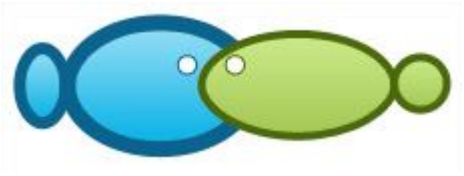

Growth of *Nannochloropsis* sp. in culture media enriched with shrub-like annual *Clerodendrum minahassae* leaf extract

Jane L. Dangeubun, Petrus P. Letsoin, Diana Y. Syahailatua

Department of Fisheries Product Technology, State Fisheries Polytechnique, Tual, Jln Langgur-Sathean Km 6, Southeast Mallucas Regency, 97611, Indonesia.
Corresponding author: J. L. Dangeubun, linda@polikant.ac.id

Abstract. This study was aimed to know the effect of water-extracted shrub *Clerodendrum minahassae* L. leaf application on the growth of algae *Nannochloropsis* sp. It used Complete Randomized Design with 4 treatments of the shrub concentrations, 2%, 4%, 6%, 8% of total water volume of *Nannochloropsis* sp. culture, positive control, and negative control, each of which with 3 replications. The culture media was water-extracted shrub *C. minahassae* leaf, whereas the positive control treatment used Walne fertilizer. Negative control treatment was also prepared without addition of Walne fertilizer or the shrub leaf fertilizer. *Nannochloropsis* sp. was cultured in the glass jar containing 1 L of water. Results showed that the highest growth of *Nannochloropsis* at 4% shrub extract concentration was $82.3x10^7 \pm 4.7x10^7$ cells in day-4, and 6% shrub extract concentration yielded the highest density in day-3, $70.7x10^7 \pm 19.0x10^7$ cells, whereas the concentration of 2%, 8%, positive control, and negative control had low cell density and quickly declined then began to die in day-4 to day-9. This finding concluded that addition of the shrub leaf concentration significantly influenced the density of *Nanochloropsis* sp. ($p < 0.05$), in which the addition of 4% and 6% shrub leaf extract concentration was the best treatment to yield the highest density of *Nannochloropsis* sp. cells.
Key Words: water-extracted shrub, glass jar, plant fertilizer, density, alga.

Introduction. Microalga is the most primitive unicellular plant commonly known as phytoplankton (Kawaroe et al 2010), a lower plant that possesses chlorophyll for photosynthesis (Rismiarti et al 2016). Phytoplankton is very interesting in marine biotechnology because of its benefits to human life (El Nabris 2012). It can be benefitted as fossil fuel substitute, such as biodiesel (Nurachman et al 2012) and bioethanol (Huang et al 2010). It is also rich in nutrients, such as fatty acid omega 3 and 6, essential amino acid (Abu-Rezq et al 2010), and producer of several carotene types (de Fretes et al 2012).

Safitri et al (2013) stated that *Nannochloropsis* sp. was one of the natural food often used for fish larvae, shrimp, clamp, zooplankton, rotifer, and *Artemia* due to its higher nutritional content than that of other microalgae, in which *Nannochloropsis* sp. holds 52.11% protein, 16% carbohydrate, 27.64% fat, 0.85% vitamin C, and 0.89% chlorophyll-*a* (Erlania et al 2010).

Nannochloropsis sp. is an industrially promising microalga that can be cultivated as alternative nutrition source due to its high productivity, protein content, and lipid composition (Hullat et al 2017). It belongs to green algae group that has greenish ball-like cell and small diameter, 2-8 µm (Kawaroe et al 2010). To obtain *Nannochloropsis* sp. content quality, it is necessary to pay attention on the environmental and nutritional condition for *Nannochloropsis* sp. requirements that can yield intact cell biomass and have high nutrition. Microalga biomass has been widely implemented in food and feed industry to obtain high economic chemical materials, such as pharma and ecological applications (Pignolet et al 2013; Borowitzka 2013; Liu et al 2016).

Microalga has 3 major applications, aquaculture, health supplement, and valuable bioproduct extraction (Sathasivam et al 2019). One of the causing factors in high natural

of microalga production is to provide culture media containing pure compound specifically made for microalga culture.

One of the phytoplankton types needed in rearing various marine fish larvae is *Nannochloropsis* sp. Its availability, either quality or quantity, is a crucial factor in larval rearing success. Seeding requires *Nannochloropsis* sp. with good quality and quantity, the cell density and high protein content (Bahua et al 2015).

The typical characteristic of *Nannochloropsis* sp. is to have cellulose-based cell wall. *Nannochloropsis* sp. contains vitamin B12 and Eicosapentaenoic acid (EPA), omega 3 HUFAs, protein, carbohydrate, lipid, vitamin C, and chlorophyll-*a*. Vitamin B12 is very important for rotifer population and EPA is good for rotifer nutrition as feed of fish larvae (Fulks & Main 1991). Microalgae are also needed as food for zooplankton (Mukhlis et al 2017). *Nannochloropsis* sp. is beneficial for rotifer *Brachionus plicatilis* to grow (Sales et al 2016; Safrizal & Humairani 2013). *Nannochloropsis* sp. culture is highly dependent upon culture media and environmental factors that influence it (Faria et al 2012; Boroh et al 2019). Major factors supporting the microalgal life are light, water, and CO_2 (Elystia et al 2019).

Flowering plant *Clerodendrum minahassae* L. is one of many plants used in Indonesia as vegetables and traditional medication for blood-enhancing drug, abdominal pain, and lung disease (Kairupan et al 2019). This study aims to develop a type of natural fertilizer made from easily found and cheap material for *Nannochloropsis* sp. culture. Application of this shrub water extract as natural fertilizer is expected to be able to increase the cell density of *Nannochloropsis* sp.

Material and Method. This study was conducted approximately for 3 months, April-June 2020 at Natural Feed Laboratory of State Fisheries Polytecnique, Tual.

Preparation of Nannochloropsis sp. culture media. Media employed in this study was seawater filtered through filter bag, then sterilized through heating up to boiling and free from microbes. Sterilization is aimed to remove or minimize the presence of microorganisms or other disrupting substances in the culture media during the study. Sterilization, according to Purnamawati et al (2012), is done to make the phytoplankton growth be uncontaminated.

Preparation of C. minahassae water extract as natural fertilizer of Walne substitute. Shrub *C. minahassae* leaf on the shoot part was cleaned, separated from the stem, washed in running water to remove the dirt, parasite, or bacteria attached on the leaf surface, drained, air-dried for 5 min. The leaves were chopped, and as much as 50 g was finely blended in 500 mL of water, filtered, and the solution was taken as *Nannochloropsis* sp. fertilizer.

Laboratory-scaled culture. The fertilizer used for laboratory culture of *Nannochloropsis* sp. was Walne fertilizer. It consists of 1 mL L^{-1} F2 and 0.5 mL L^{-1} of vitamin mix (Gusrina 2008). The culture was carried out in the controlled glass jar using F2 fertilizer at a dose of 1 mL L^{-1} and vitamin mix of 0.5 mL L^{-1} (Figure 1). *Nannochloropsis* sp. was reared in the controlled glass jar containing 1 L of water. The inoculant used was 30% of total culture volume, then placed in the culture cupboard facilitated with 2 units of 40 watt-Philip TL lamp as light source and aerated to supply oxygen. *Nannochloropsis* sp. needs light intensity between 2,500 and 5,000 lux. It is in line with Inthe (2012) who utilizes the light intensity of 3,000-10,000 lux. The observation on cell growth was daily carried out until the harvest at the 4^{th} day. Harvest was conducted through total harvest system used as inoculants for larger volume culture.

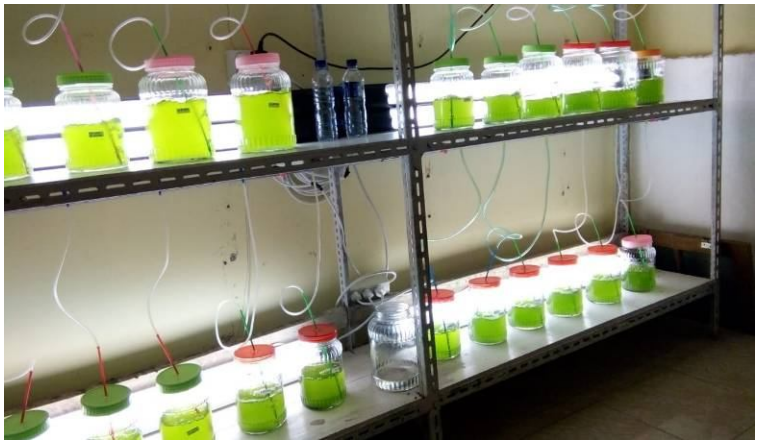

Figure 1. Laboratory-scaled *Nannochloropsis* sp. culture.

Shrub leaf extract treatment as natural fertilizer for Nannochloropsis sp. growth. This experiment applied 4 treatments with 3 replications, positive control using Walne fertilizer, and negative control without any fertilizer. The water-extracted shrub leaf used in this study was at the concentration of 2%, 4%, 6%, 8%, positive control, and negative control with 700 mL of sterile seawater at the salinity of 27 ppm and 300 seeds of *Nannochloropsis* sp. Each glass jar that contains sterile seawater and *Nannochloropsis* sp. seed was added with water extract of the shrub leaf, except positive control and negative control. Observations on *Nannochloropsis* sp. cell growth were conducted daily using microscope facilitated with haemocytometer. Water quality parameters measured were temperature, salinity, and acidity (pH). These observations were carried out until the growth of *Nannochloropsis* sp. showed declining trend or mortality phase.

The estimation of *Nannochloropsis* sp. growth followed Isnansetyo & Kurniastuty (1995):

$$\text{Cell density (cells mL}^{-1}\text{)} = n \times 4 \times 10^6$$

where n = number of cells, and 4×10^6 = heamocytometer constant

The effect of different treatment concentrations on the growth rate was analyzed using Complete Randomized Design at 95% confidence (a = 0.05) (Nurhatika 2010). Data processing was done using ver. 17 SPSS software. The outputs are presented in tables.

Results and Discussion

Nanochloropsis sp. growth. Observation on *Nannochloropsis* sp. was conducted daily for 7 days. The growth curve of lag phase (adaptation) occurred in day-1, in which all treatment concentrations, 2%, 4%, 6%, 8%, positive control, and negative control had *Nannochloropsis* sp. cell development, whereas in day-2, all treatments showed cell development, except negative control, because it ran out of nutrients in the media from no fertilization and used only the limited nutrients in the seawater that results in mortality of *Nannochloropsis* sp. cells. In this phase, the algae still adapted to the environment, in which the concentration of 2%, 4%, 6%, 8%, and positive control yielded gradual increase in number of cells since *Nannochloropsis* sp. could adapt to the new environment. Different number of *Nannochloropsis* sp. cells at each treatment could result from different adaptability of *Nanochloropsis* sp. cells to the new medium (Fadilla 2010).

Cell growth of *Nanochloropsis* sp. during the study showed that addition of the shrub leaf extract concentration significantly influenced the density of *Nannochloropsis* sp. ($p < 0.05$). Addition of 4% extract concentration gave the highest density at day-4, $82.3 \times 10^7 \pm 4.7 \times 10^7$, then the density started declining in day-5 to day-9, whereas addition of 6% extract concentration yielded the highest cell density at day-3, $70.7 \times 10^7 \pm 19.0 \times 10^7$, then the density declined in day-4 to day-9. The extract

concentration treatment of 2%, 8%, positive control, and negative control did not give significant growth, and *Nanochloropsis* sp. cell density started declining in day-4 to day-9, in which the number of cells became very few up to reaching the mortality phase (Figure 2).

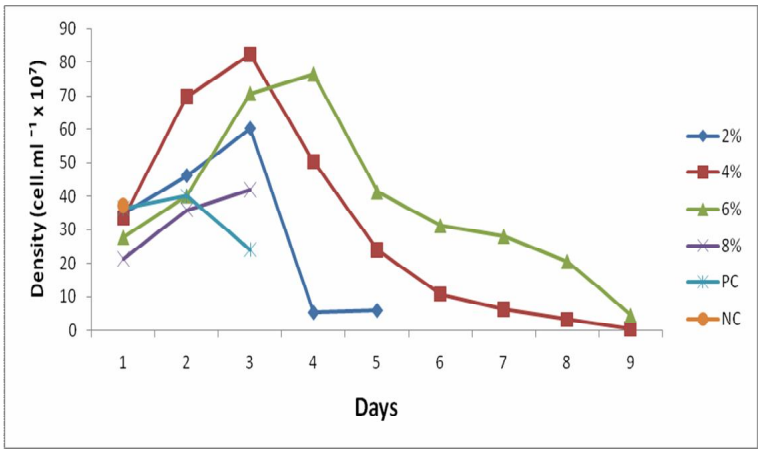

Figure 1. Nanno cell growth.

The extent of growth is used as a measure of the media carrying capacity on the algal growth. The irregular growth curve of each treatment could result from different adaptability of *Nanochloropsis* sp. cells to the new medium. The organisms run the metabolism but cell division has not significantly occurred so that the cell density has not developed yet (Fadilla 2010). Cell mortality could result from insufficient availability of nutrients in the media that affects the cell density of *Nanochloropsis* sp. Water salinity, temperature, and pH of the culture media were in the range of 27-28 ‰, 26-27^{o}C, and 7.7-8, respectively. It means that water quality parameters in the culture media are in normal range and do not directly influence the cell growth. These findings are supported by Sari et al (2012) that type and level of nutrients in the media highly affect the growth of *Nannochloropsis* sp.

Faster density decline in the concentration of 2%, 8%, positive control, and negative control (Figure 1) could result from that the nutrients in *Nanochloropsis* sp. water extract as culture media have been not enough. It is in agreement with Sutomo (2005) that algae are not capable of benefitting nutrients in limited number condition so that the population density could decline. The cell growth of *Nanochloropsis* sp. during the study experienced several phases as follows:

1) Lag phase as the first phase of the microalga growth in culture. This phase occurs when the inoculum is placed into the culture media, and microalga *Nanochloropsis* sp. makes an adaptation to the new media to grow before the cell division occurs. All treatment concentrations cause the microalgae pass through this phase with slow growth and few cell divisions.

2) Exponential phase. In this phase, the microalgae have very fast growth due to increased photosynthetic activity and yield high biomass (Madigan et al 2010). During this period, the cultured microalgae are in stable condition and the cell division occurs faster. The exponential phase started in day-2 to day-4 at the treatment of 4% extract concentration, day-1 to day-6 at 6% extract concentration, day-2 to day-4 at 2% and 8% extract concentration, respectively. Increase in cell growth is highly dependent upon the availability of nutrients in the culture media, whereas decline in cell density could be caused by insufficient nutrient availability in the medium due to no nutrient addition and space competition among the cells in the limited volume (Musa et al 2013). This exponential phase makes the cell structure be in normal condition and there is an equilibrium between nutrients in the medium and in the cell.

3) Cell growth decline phase. Decline in number of cells occurs because of high nutrient competition in the culture media and the nutrients are not enough available for the fast growing population in the exponential phase. The cell density at the treatment of

2% extract concentration started declining in day-4 to day-5, whereas in day-5 to day-9, no *Nanochloropsis* sp. cells were recorded because all were dead. Cell density declined in day-5 to day-9 at the treatment of 4% extract concentration, day-6 to day-9 at the treatment of 6%, while at the treatment of 8% extract concentration, positive control, and negative control the cell growth started declining in day-3 to day-4, and no *Nanochloropsis* sp. was recorded afterwards since all cells were dead. The imbalance between number of nutrients and microalga population makes a part of the nano microalgae do not gain enough nutrition for division process.

4) In mortality phase, number of microalga *Nanochloropsis* sp. cells declines until the death, in which there is no any *Nanochloropsis* sp. cell in the treatment media. The application of 2%, 8%, and positive control have led to death in day-3 to day-4, whereas 4% and 6% treatment concentrations showed decline in number of cells in day-5 to day-9. It is shown by *Nanochloropsis* sp. cell mortality since water quality changes to worse condition, nutrient content in the culture medium declines, metabolism ability of the microalga decreases from insufficient nutrient content, and culture media is limited, so that their cell division ability tends to be restricted as well, and then the cell division will stop due to insufficient nutrients in the culture media. According to Sari et al (2012), the type of nutrients and the concentration in the media highly influence the growth of *Nannochloropsis* sp. It is in line with Nurfadillah et al (2010) that decline in phytoplankton growth results from several factors, such as photosynthesis, sufficient nutrient availability, and turbidity.

Statistical analysis (Table 1) showed that addition of the shrub leaf extract concentration gave significantly different density of *Nanochloropsis* sp. during the study ($p < 0.05$). *Nannochloropsis* sp. density reached the highest cell density in day-4, $82.3x10^7 \pm 4.7x10^7$ and then declined in day-5 to day-9, while treatment of 6% yielded the highest cell growth in day-3, $70.7x10^7 \pm 19.0x10^7$, then declined in day-4 to day-9. Compared with 4 other treatments, the extract concentration of 2%, 8%, positive control, and negative control did not have significant growth and the cell density started falling down in day-4 to day-9, then went into mortality phase (Table 1).

Table 1

Density of *Nannochloropsis* sp. during the study period

Day	*Treatments*					
	2%	*4%*	*6%*	*8%*	*Positive control (PC)*	*Negative control (NC)*
1	$34.8x10^7 \pm 2.4x10^{7ab}$	$33.6x10^7 \pm 1.8x10^{7b}$	$27.6x10^7 \pm 1.4x10^{7c}$	$21.3x10^7 \pm 2.3x10^{7d}$	$36.4x10^7 \pm 0.4x10^{7ab}$	$37.3x10^7 \pm 1.7x10^{7a}$
2	$46.0x10^7 \pm 4.5x10^{7ab}$	$50.4x10^7 \pm 2.1x10^{7a}$	$40.0x10^7 \pm 0.00^{bc}$	$36.0x10^7 \pm 0.00^{c}$	$40.3x10^7 \pm 11.0x10^{7bc}$	00.00 ± 0.00^{d}
3	$60.1x10^7 \pm 2.2x10^{7ab}$	$69.7x10^7 \pm 5.0x10^{7a}$	$70.7x10^7 \pm 19.0x10^{7a}$	$42.0x10^7 \pm 2.0x10^{7b}$	$18.9x10^7 \pm 20.1x10^{7c}$	00.00 ± 0.00^{c}
4	$5.3x10^7 \pm 9.2x10^{7c}$	$82.3x10^7 \pm 4.7x10^{7a}$	$41.2x10^7 \pm 38.3x10^{7b}$	00.00 ± 0.00^{c}	00.00 ± 0.00^{c}	00.00 ± 0.00^{c}
5	$5.8x10^7 \pm 1.0x10^{7b}$	$24.0x10^7 \pm 20.8x10^{7ab}$	$38.3x10^7 \pm 33.3x10^{7a}$	00.00 ± 0.00^{b}	00.00 ± 0.00^{b}	00.00 ± 0.00^{b}
6	00.00 ± 0.00^{b}	$10.7x10^7 \pm 10.1x10^{7b}$	$31.1x10^7 \pm 26.9x10^{7a}$	00.00 ± 0.00^{b}	00.00 ± 0.00^{b}	00.00 ± 0.00^{b}
7	00.00 ± 0.00^{b}	$6.3x10^7 \pm 5.6x10^{7b}$	$27.9x10^7 \pm 24.8x10^{7a}$	00.00 ± 0.00^{b}	00.00 ± 0.00^{b}	00.00 ± 0.00^{b}
8	00.00 ± 0.00^{b}	$3.2x10^7 \pm 2.8x10^{7b}$	$20.5x10^7 \pm 18.2x10^{7a}$	00.00 ± 0.00^{b}	00.00 ± 0.00^{b}	00.00 ± 0.00^{b}
9	00.00 ± 0.00^{b}	$4.0x10^6 \pm 0.4x10^{7b}$	$4.4\ x10^7 \pm 4.8x10^{7a}$	00.00 ± 0.00^{b}	00.00 ± 0.00^{b}	00.00 ± 0.00^{b}

Notes: a – day-2 and 4 for 4%, day-3, 5, 6, 7, 8, 9 for 6%, day-1 for NC; b – day-5, 6, 7, 8, 9 for 2%, day- 4 for 6%, day-5, 6, 7, 8, 9 for 8%, PC and NC; ab – day-1, 2, 3 for 2%, day-5 for 4%, day-1 for PC; bc – day-2 for 6%, day-1 for PC; c – day-4 for 2%, day-1 and 3 for 8%, day-4 for PK, day-3 for NC; d – day-8 for 8%, day-2 for NC.

The growth of *Nannochloropsis* sp. is not only affected by the nutrient content but the environmental condition as well, such as water temperature, salinity, and pH. During the study, water quality was in the normal range, temperature of 26-27°C, salinity of 27-28 ‰, and pH of 7.7-8 (Table 2). According to Sahira et al (2017), *Nannochloropsis* sp. needs water temperature of 26-28°C, salinity of 25-26 ppt, and pH of 7-8 to grow, and Jadid et al (2017) found the optimum salinity range of 23-27 ‰, temperature of 28-30.7°C, and pH of 7-8.9, but Pal et al (2011) claimed that *Nannochloropsis* sp. can grow at various salinity levels. Thus, the water quality measurements recorded in this study are in normal condition, even though the treatment concentrations of 2%, 6%, 8%, NC, and PC yield low *Nannochloropsis* sp. cell density. It indicates that sufficient nutrient availability needed by *Nannochloropsis* sp. for the growth is not fulfilled since there is no equilibrium between nutrient availability and number of cells in the culture media. The treatment concentration of 4% water extract of *C. minahassae* leaf could give sufficient nutrient for *Nannochloropsis* sp. growth than other treatments. The type and level of nutrient in the media highly influences the growth of *Nannochloropsis* sp. (Sari et al 2012).

Table 2

Water quality measurements in *Nannochloropsis* sp. culture media

Parameters	*2%*	*4%*	*6%*	*8%*	*PC*	*NP*	*Quality standard*
Temperature (°C)	26	27	27	27	27	27	28-30.7 (Jadid et al 2017)
Salinity (‰)	27	28	27	27	28	27	23-27 (Jadid et al 2017)
pH	8	7.8	7.7	7.8	7.7	7.8	7-8.9 (Jadid et al 2017)

According to Sylvester et al (2002), the optimum range of salinity for microalga growth is 25-35%. Salinity of the culture media increases from evaporation caused by the temperature of the light used during the culture experiment. The salinity change could also result from water agitation in the culture media that results in evaporation. The acidity level (pH) of the culture media ranged 7-8 during the study, and this condition occurs in optimum range.

Conclusions. Addition of shrub-like annual leaf concentration gave significant effect on *Nanochloropsis* sp. density ($p < 0.05$). Addition of 4% shrub-like annual extract yielded the highest density at the third day, $82.3x10^7 \pm 4.7x10^7$, and addition of 6% concentration gave the highest density at the fifth day, $70.7x10^7 \pm 19.0x10^7$.

Acknowledgements. We thank the Head of Directorate of Research and Community Engagement, Deputy of Research Strengthening and Development Department of the Ministry of Research and Technology and National Innovation that has provided the research grant. This appreciation is also addressed to the UPPM of Politeknik Perikanan Negeri Tual for the involvement in this study.

References

Abu-Rezq T. S., Al-Hooti S., Jacob D., Al-Shamali M., Ahmed A., Ahmed N., 2010 Induction and extraction of α-carotene from the locally isolated *Dunaliella salina*. Journal of Algal Biomass Utilisation 1(4):58-83.

Bahua H., Hendrawan Y., Yulianingsih R., 2015 [Effect of auxin synthetic naftalena acetic acid on the growth of microalga (*Nannochloropsis oculata*)]. Jurnal Keteknikan Pertanian Tropis dan Biosistem 3(2):179-186. [in Indonesian]

Boroh R., Litaay M., Umar M., Ambeng, 2019 [Growth of *Chlorella* sp in several combinations of culture media]. BIOMA: Jurnal Biologi Makassar 4(2):129-137. [in Indonesian]

Borowitzka M. A., 2013 High-value products from microalgae-their development and commercialisation. Journal of Applied Phycology 25:743-756.

de Fretes H., Susanto A. B., Prasetyo B., Limantara L., 2012 [Caretenoids from macroalgae and microalgae healt potential, applicattion and biotechnology]. Jurnal Teknologi dan Industri Pangan 23(2):221-228. [in Indonesian]
El Nabris K., 2012 Development of cheap and simple culture medium for the microalgae *Nannochloropsis sp.* based on agricultural grade fertilizers available in the local market of Gaza Strip (Palestine). Journal of Al Azhar University-Gaza (Natural Sciences) 14:61-76.
Elystia S., Muria R. S., Pertiwi S. I. P., 2019 [The use of microalga *Chlorella* sp. for lipid production in Hotel liquid waste media in C:N ratio variations and light wavelength]. Jurnal Sains dan Teknologi Lingkungan 11(1):25-43. [in Indonesian]
Erlania, Widjaja F., Adiwilaga E. M., 2010 [Instant rotifer (*Brachionus rotundiformis*) storage at different temperatures, feeding with concentrate microalgae]. Jurnal Riset Akuakultur 5(2):287-297. [in Indonesian]
Fadilla Z., 2010 [Effect of liquid waste of tofu on microalga *Scenedesmus* sp. growth]. BSc Thesis, Fakultas Sains dan Teknologi, Universitas Islam Negeri Syarif Hidayatullah, Jakarta, 65 pp. [in Indonesian]
Faria G. R., Paes C. R. P. S., Castro D. J. F. A., Tinoco N. A. B., Barbrino E., Lourenco S. O., 2012 Effects of the availability of CO_2 on growth, nutrient uptake, and chemical composition of the marine microalgae *Chlorella* sp. and *Nannochloropsis oculata*, two potentially useful strains for biofuel production. International Research Journal of Biotechnology 3(5):65-75.
Fulks W., Main K. L., 1991 The design and operation of live feeds production systems. In: Rotifer and microalgae culture systems. Proceedings of a US-Asia Workshop, Honolulu, Hawaii, January 28-31, 1991, The Oceanic Institute, Hawaii, USA, pp. 3-52.
Gusrina, 2008 [Fish culture part 3 for Vocational High School]. Direktorat Pembinaan Sekolah Menengah Kejuruan, Direktorat Jenderal Manajemen Pendidikan Dasar dan Menengah dan Departemen Pendidikan Nasional Tahun 2008, Jakarta, pp. 352-357. [in Indonesian]
Huang G., Chen F., Wei D., Zhang X., Chen G., 2010 Biodiesel production by microalgal biotechnology. Applied Energy 87(1):38-46.
Hullat C. J., Wijffels R. H., Bolla S., Kiron V., 2017 Production of fatty acids and protein by *Nannochloropsis* in flat-plate photobioreactors. PLoS One 12(1):e0170440.
Inthe I. C. E., 2012 [Effect of lighting on *Nannochloropsis* sp. biomass production on flat plate reactor]. BSc Thesis, Fakultas Teknik, Universitas Indonesia, Program Studi Teknologi, Biopress Depok, 107 pp. [in Indonesian]
Isnansetyo A., Kurniastuty, 1995 [Phytoplankton-zooplankton culture technique. Natural feed for marine organism hatchery]. Kanisius, Yokyakarta, 116 pp. [in Indonesian]
Jadid R., Dewiyanti I., Nurfadillah, 2017 [Addition of coconut water in growth media of *Nannochloropsis* sp.]. Jurnal Ilmiah Mahasiswa Kelautan dan Perikanan Unsyiah 2(1):113-118. [in Indonesian]
Kairupan C. F., Mantiri F. R., Rumende R. R. H., 2019 Phytochemical screening and antioxidant activity of ethanol extract of leilem (*Clerodendrum minahassae* Teijsm. & Binn) as an antihyperlipidemic and antiatherosclerotic agent. IOP Conference Series: Earth and Envirnmental Science, Manado 217:012016.
Kawaroe M., Prartono T., Sanuddin A., Wulansari D., Augustine D., 2010 [Microalga: potency and its utilization for biofuel production]. Penerbit IPB Press, Bogor, 150 pp. [in Indonesian]
Liu L., Pohnert G., Wei D., 2016 Extracellular metabolites from industrial microalgae and their biotechnological potential. Marine Drugs 14(10):191.
Madigan M. T., Martinko J. M., Stahl D. A., Clark D. P., 2010 Brock biology of microorganisms. 13th edition, Benjamin Cummings, 1152 pp.
Mukhlis A., Abidin Z., Rahman I., 2017 [The effect of ammonium sulphate fertilizer concentration on the population growth of *Nannochloropsis* sp. cells]. Jurnal Biowallacea 3(3):149-155. [in Indonesian]

Musa B., Raya I., Dali S., 2013 [Effect of Cu_2^+ ion addition on the growth rate of phytoplankton *Chlorella vulgaris*]. Research Report, Fakultas MIPA, Universitas Hasanuddin, Makassar, 9 pp. [in Indonesian]
Nurachman Z., Hartati, Anita S., Anward E. E., Novirani G., Mangindaan B., Gandasasmita S., Syah Y. M., Panggabean L. M. G., Suantika G., 2012 Oil productivity of the tropical marine diatom *Thalassiosira* sp. Bioresource Technology 108:240-244.
Nurfadillah, Damar A., Adiwilaga E. M., 2010 [Community of phytoplankton in lake Laut Tawar, Aceh Province]. Jurnal Depik 1(2):93-98. [in Indonesian]
Nurhatika S., 2010 [Complete randomized design]. Departemen Biology, Fakultas Matematika dan Ilmu Pengetahuan Alam, Institut Teknologi Sepuluh November, Surabaya (unpublished), 18 pp. [in Indonesian]
Pal D., Khozin-Goldberg I., Cohen Z., Boussiba S., 2011 The effect of light, salinity, and nitrogen availability on lipid production by *Nannochloropsis* sp.. Applied Microbial and Cell Physiology 90:1429-1441.
Pignolet O., Jubeau S., Vaca-Garcia C., Michaud P., 2013 Highly valuable microalgae: biochemical and topological aspects. Journal of Industrial Microbiology & Biotechnology 40:781-796.
Purnamawati F. S., Soeprobowati T. R., Izzati M., 2015 [The potential of *Chlorella vulgaris* Beijerinck in laboratory-scaled heavy metal Cd and Pb remediation]. Jurnal Bioma 16(2):102-113. [in Indonesian]
Rismiarti A., Kusumaningrum H., Zainuri M., 2016 [Characterization and molecular identification of fusan from interspecific protoplast fussion of *Chlorella pyrenoidosa* and *C. vulgaris* using 18SrDNA]. Jurnal Bioma 18(2):30-40. [in Indonesian]
Safitri M. E., Diantari R., Suparmono, Muhaemin M., 2013 [Total fat content of *Nannochloropsis* sp. at different photoperiods]. Jurnal Rekayasa dan Teknologi Budidaya Perairan 1(2):128-134. [in Indonesian]
Safrizal E., Humairani R., 2013 [Growth rate stimulation of rotifer (*Brachionus plicatilis*) population after added with feed in the treatment media]. Jurnal Lentera 13:81-88. [in Indonesian]
Sahira, Muskita W. H., Astuti O., 2017 The effect of nitrophoska fertilizer dose on *Nannochloropsis* sp growth. Jurnal Media Akuatika Vol.2 (4): 494-501, 2017. ISSN 2503-4324
Sales R., Mélo R. C. S., de Moraes Jr. R. M., da Silva R. C. S., Cavalli R. O., Navarro D. M. A. F., de Souza Santos L. P., 2016 Production and use of a flocculated paste of *Nannochloropsis oculata* for rearing newborn seahorse *Hippocampus reidi*. Algal Research 17:142-149.
Sari A. S. P., Wisanti, Ratnasari E., 2012 [Effect of different fertilizer application on the population growth rate and fat content of *Nannochloropsis oculata*.] LenteraBio 1(1):55-61. [in Indonesian]
Sathasivam R., Radhakrishnan R., Hashem A., Abd_Allah E. F., 2019 Microalgae metabolites: a rich source for food and medicine. Saudi Journal of Biological Sciences 26(4):709-722.
Sutomo, 2005 [Cultivation of 3 types of algae (*Tetraselmis* sp., *Chlorella* sp. and *Chaetoceros gracilis*) and the effect of initial density on the growth of *C. gracilis* in the laboratory]. Oseanologi dan Limnology di Indonesia 37:45-58. [in Indonesian]
Sylvester B., Nelvy, Sudjiharno, 2002 [Phytoplankton biology, phytoplankton and zooplankton culture]. Balai Budidaya Laut Lampung. Makara Teknologi 9:3-23. [in Indonesian]

Received: 07 September 2020. Accepted: 02 October 2020. Published online: 21 October 2020.
Authors:
Jane Lulinda Dangeubun, Department of Fisheries Product Technology, State Fisheries Polytechnique, Tual, Jln Langgur-Sathean Km 6, Southeast Mallucas Regency, 97611, Indonesia, e-mail: linda@polikant.ac.id
Petrus Paulus Letsoin, Department of Fisheries Product Technology, State Fisheries Polytechnique, Tual, Jln Langgur-Sathean Km 6, Southeast Mallucas Regency, 97611, Indonesia, e-mail: p2_letsoin@polikant.ac.id
Diana Y. Syahailatua, Department of Fisheries Product Technology, State Fisheries Polytechnique, Tual, Jln Langgur-Sathean Km 6, Southeast Mallucas Regency, 97611, Indonesia, e-mail: yulasyahailatua@yahoo.co.id

How to cite this article:
Dangeubun J. L., Letsoin P. P., Syahailatua D. Y., 2020 Growth of *Nannochloropsis* sp. in culture media enriched with shrub-like annual *Clerodendrum minahassae* leaf extract. AACL Bioflux 13(5):2807-2815.