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The Growth of *Chlorella vulgaris* Cultured in Liquid Organic Fertilizer of Water Hyacint H (*Eichhornia crassipes*) at Different Salinities

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Abstract

Wa Iba, Citra Utami and Abdul M. Balubi. 2019. The Growth of Chlorella vulgaris Cultured in Liquid Organic Fertilizer of Water Hyacint H (Eichhornia crassipes) at Different Salinities, Indonesia Aquacultura Indonesiana, 20 (2) : 117-126. This study aims to determine the effect of salinity on the growth of microalgae *C. vulgaris* cultured in 5% concentration of water hyacinth liquid organic fertilizer. The research used a completely randomized design with four salinity treatments at 30, 35, 40 and 45 ppt in triplicates. The volume of culture media was 150 mL that consisted of 142.5 ml of sterile sea water and 7.5 ml of water hyacinth organic fertilizer. The increase in microalgae cell density was observed every other day using haemocytometer under light microscope. The culture was harvested after they reached stationary phase at day 8. Culture age ($p= 0,00<0.05$) and salinity ($p= 0,00<0.05$) affected the growth, dry weight and biomass productivity of *C. vulgaris*. Cells density and yield in all salinities tested was significantly different ($p= 0,00<0.05$) at stationary phase of growth. Higher cell density, specific growth rate, yield, dry weight and biomass productivity were observed in 40 ppt salinity compared to other salinities. At 40 ppt, cell density was 85.33 x 10^4 cells. ml^{-1} as well as specific growth rate that was at 0.839 $cells.day^{-1}$, with the highest average yield of *C. vulgaris* was observed on day 6 and day 8 (exponential to stationary phase) at 115, 3 x 10^4 $cells.ml^{-1}$. Similarly, considerably higher dry weight at 40 ppt was observed at 0.038 $g.L^{-1}$ with biomass productivity at 0.032 $g.L^{-1}.day^{-1}$ compared to other salinities tested However these results were not significantly different ($p=0.7>0.05$) from other salinities. This study suggested that 40 ppt can be used to culture *C. vulgaris* in 5 % concentration of water hyacinth organic medium to obtain better growth and higher biomass production.

Keywords : *Chorella vulgaris*, salinity, liquid organic fertilizer, growth, biomass.

Introduction

Microalgae is a group of microscopic aquatic plants that contain chlorophyll making them a very efficient factory in capturing and utilizing solar energy and CO_2 for photosynthesis. Microalgae biomass contains important nutrients such as protein, carbohydrates and lipid. The percentage of these components varies depending on the microalgae species (Kawaroe et al., 2010). Microalgae has been used to support various industries such as aquaculture, energy and functional food. They are a source of protein in shrimp or fish larvae cultivation, food supplements for humans, and in pharmacology, they are used as antibacterial, antioxidant, and antiviral (Umbu et al., 2014) as well as feedstocks for biofuel and bioremediation (Setyaningsih et al., 2015).

Chlorella vulgaris is a photosynthetic and eukaryotic microalgae of the Chlorellaceae family. This organism is a unicellular green microalgae, has round cells with a diameter of 2-10 µm with asexual reproduction where the stem cells reproduce 4 daughter cells, making growth rate is higher (the time to multiply the cell is about 19 hours) (Dalir*yet al.*, 2017).The

pattern of microalgae growth in batch photobioreactors forms a sigmoid curve consisting of four phases, the linear (lag phase), exponential, stationary, and death phase (Prayitno, 2015).

Salinity is an important paramaters affecting the level of cell division which in turn will affect the biomass and productivity of microalgae in both in natural and controlled condition (Asia et al., 2018). High salinity in culture medium aims to create stress conditions that can accelerate the growth of microalgae (Setyaningsih et al., 2013). In the other hand, appropriate or optimal culture salinity will create a balance in osmotic pressure between microalgae cells and culture media thus the growth and development of microalgae will be optimal (Ratri and Hermana, 2013). Salinity is also an important factor in pigment formation (Adenan, 2013). Culturing microalgae in normal or above seawater salinity is more preferred in the face of climate change due to decreasing of freshwater availability.

Microalgae biomass production in is the main goal for the cultivation of microalgae (Oktovianus, 2018). Therefore, the appropriate culture medium is very important in the growth of microalgae in controlled condition include for *Chorella*sp. If the intake of nutrients and medium is not sufficient, the growth rate will be hampered. Microalgae culture in semi-open systems with a semi-mass scale requires constant supply of nutrients (i.efertilizers) in their culture media to maintain the quantity, quality and stability of microalgae cell production thus fertilizer selection depends on nutrient composition needed in microalgae culture (Suminto, 2009). Therefore, the culture medium must contains various nutrients needed for microalgae growth and development. The medium needed for the development of *Chorella*sp. is relatively simpler and only requires fewer types of nutrients compared to the medium for other microalgaespceis. There are several media that usually used for *Chorella*culture, includeBenneck, Detmer, commercial fertilizer and Walnemedium (Najmuddin, 2011). However, those commercial fertilizer is considerably expensive and not readily available particularly in remote region of tropical developing countries. Culture media, thus, remains a bottleneck for mass culture of microalgae at commercial scale. Therefore, searching a cheap and more readily avalaible culture medium is a priority for mass culture microalgae in the tropics.

Hyacinth (*Eichorniacrassipes*) is a weedy floating aquatic plant that highly competitive and may cause several problems in aquatic environment. However, because of their ability to utilize nitrogen (N) and phosphorous (P) efficiently in water, water hyacinth can be used as organic fertilizer as the accumulative nutrients may beneficial for agricultural plants (Shawwal, 2010). The use of *E. crassipes*as fertilizer for agriculture and aquacultureis well known (Sanni and Adesina, 2011; Osoro et al., 2014; John, 2016; Ravi et al., 2019) and produced better growth and yield of angelfish at lower costs than inorganic fertilizer (Sipauba-Tavares et al., 2017).Water hyacinth in form of liquid organic fertilizer at 5 % concentration that was used as culture medium for *C. vulgaris*at normal seawater salinity has been investigated with promising results (Goa et al., 2019). However, the effect of salinity on the production of *C. vulgaris* using water hyacinth is not yet known despite the importance of salinity factor for stimulating growth of the microalgae. Therefore, this present study investigates the effect of salinity on growth and biomass productivity of C. vulgaris cultured in media that contains water hyacinth liquid organic fertilizer.

Materials and Methods

Water Hyacinth Liquid FertilizerPreparation

Liquid organic water hyacinth fertilizer was prepared according to the procedure by Ayu (2018) and Moi et al. (2015). Leaves and trunks of the plant were cut into small pieces and cleaned from debris and dirt by washing it in freshwater. The cleaned pieces of water hyacinth were then fermented in 20 mL Effective Microorganism (EM4) probiotic and sugar in 1 L of water.

The fermentation was mixed thoroughly every day and after 4 weeks, the fermented water hyacinth was filtered, sterilized and keep it in covered glass bottles at room temperature for further use. The nitrogen and phosphorous conteent of water hyaconth liquid fertilizer was analyzed before used in the experimet.

Microalgae Stock Culture Preparation

Intial stock of *C. vulgaris*was obtained from the Brackish Aquaculture and Fisheries Center, Mappakalompo Village, Takalar, South Sulawesi. Upon arrival, the stock was cultured and acclimatized in f/2 culture media with salinity of 30, 35, 40 and 45 ppt respectively. Acclimatization is aimed to adapt the microalgae in respective salinity that will be applied in the culture experiment.

Culture Experiment

The culture media was prepared using 5% or 7.5 ml of water hyacinth liquid fertilizer in 142.5 ml of sterile sea water. The culture medium was prepared in 250 ml sterilized erlenmeyerin triplicates for each salinity tested. Commercial salt was used to increase the salinity of culture medium to 30, 35, 40 and 45 ppt. Initial density of microalga culture was 10 x 10^4 cell.mL^{-1} and gentle mixing once a day. The culture was maintaned at 10 µmol photons m^{-2} s^{-1} using 10 watt table lamp under 12:12 photoperiod.

The density of cultured microalgae in each replicates was measured every two days using Neubaeurhaemocytometer under light microscope . The culture was harvested after 8 days during late stationary phase.Cultured microalgae at each salinity were filtered using pre-weighed, pre-combusted (1 h, 100 ± 5°C) GF/F filters (Ɵ = 47 mm), and rinsed with distilled water to eliminate salts. Filters were oven dried at 100±5°C for 1 h, placed overnight over $KMnO_4$ salts in a vacuum desiccator, and weighed to obtain dry weight of the algae.

Data Analysis

Growth rate of *C. vulgaris*

Specific growth rate (µ) of *C. vulgaris* was calculated using the following formula from Moheimani et al. (2013):

$$\mu = \frac{Ln\left(\frac{Ni}{N0}\right)}{ti - t0}$$

Production (Yield) of Microalgae

Obervation on final cell density was calculated based on average final density during stationary phase. The yield of *C. vulgaris* in this study was calculated based on formula in Iba et al. (2018) as follows:

$$Yield = \frac{(N1 + N2)}{2}$$

Determination of Dry Weight (DW)

Dry weight (DW) is determined by the equation referring to the following formula by Moheimani et al. (2013):

$$DW = \frac{Filter\ weight\ plus\ microalgae\ (g) - filter\ weight\ (g)}{Sample\ volume\ (L)}$$

Biomass Productivity

The biomass productivity of *C. vulgaris*was calculated by the equation that refers to Crismadha (1993):

$$Biomass\ Productivity = DW\ x\ \mu\ during\ exponential\ phase$$

Growth data were statistically analysed using repeated measures analysis of variance (ANOVA). Whereas yield, dry weigt and biomass productivity data were analyzed using one-way ANOVA. Multiple comparisons among treatment means were made with the Duncan multiple comparison test using the Statistical Analysis Software Program of SPPS 16.0 for Windows. Results were considered statistically significant at the level of $p<0.05$

Results

Nutrient Content of Water Hyacinth Liquid Fertilizer

Nitrogen content of 5 % water hyacinth liquid fertilizer used in this was higher (225 ppm) compared to phosphorous (9.6 ppm) (Table 1)

Table 1. N and P content of water hyacinth liquid fertilizer

No.	Nutrient	Full concentration (ppm)	Concentration in 150 ml (ppm)	Concentration in 1 L (ppm)
1	Nitrogen	5100	225	1500
2	Phosphat	192,286	9,6	64

Growth of C. vulgaris

C. vulgaris cell growth experienced lag phase or adaptation when cultured at 30, 35 and 40 and 45 ppt salinity. Exponential phase of growth was oberved on day 2 to 4 with cell density at 40ppt was higher than other salinties tested. This trend was consistently observed until late stationary phase where microalgae density was 85.33 x 10^4 cells.ml^{-1} when cultured at 40 ppt whereas at 30 and 35 ppt were 70.53 x 10^4 cells.ml^{-1} and 71.13 x 10^4 cells.ml^{-1}, respectively. Late stationary phase was observed at day 8 of the culture for all salinities tested except for 45 ppt. *C. vulgaris* density at 45 ppt was lower compared to all salinities tested (53.73 x 10^4 cells. ml^{-1}). Growth of *C. v*ulgaris declined sharply at at all salinities on day 10 indicating that death phase was reached (Fig. 1).

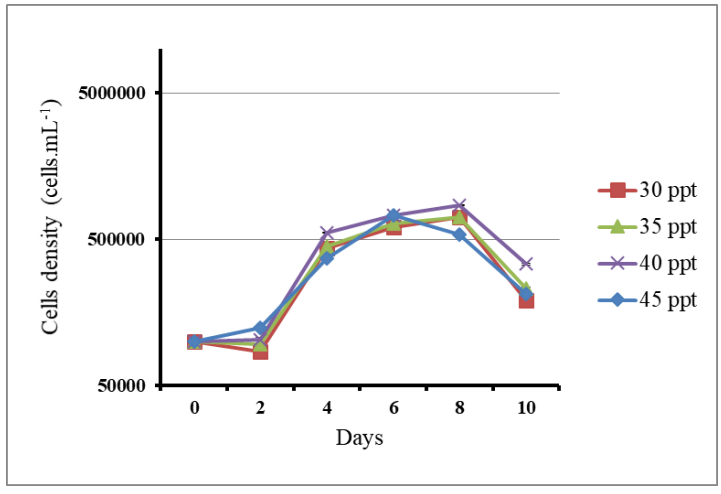

Figure 1. Growth of *C. vulgaris* in 5 % water hyacinth liquid fertilizer at different salinities *(*mean ± SE)

The results of the repeated measures ANOVA showed that intercation between days of culture and salinity significantly affected the growth of *C. vulgaris* during the study (p= 0.00>0.05). Duncan test confirmed that the cells density of *C. vulgaris* at 30 ppt was significantly different from the 35, 40 and 45 ppt salinity tested at different days of culture.

Higher specific growth rate of *C. vulgaris* cultured at 40 ppt was observed during exponential phase of growth compared to other salinities tested (Fig. 2). Average growth rate of *C. vulgaris*at 40 pptwas 0.839

cells.day^{-1}whereas at 30, 35 and 45 ppt was 0.817, 0.772 and 0,549cells.day^{-1}, respectively.

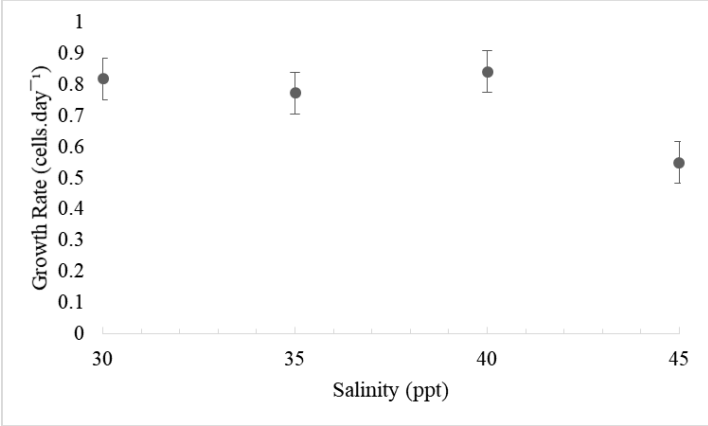

Figure 2. Growth rate of *C. vulgaris*during (mean ± SE) at different salinities.

ANOVA results showed that salinity significantly affected growth of *C. vulgaris* during logaritmic phase (p = 0,00<0.05). The lowest growth rate was observed at 45 ppt wehereas the highest growth was at 40 ppt salinity. Similar growth rate was exhibited when *C. vulgaris* cultured at 30 and 35 ppt.

Yield of C. Vulgaris

The highest yield during stationary phase was produced when *C. vulgaris* was cultured at 40 ppt with 1,2 x 10^6 cells.ml^{-1} when compared to other salinities tested. At 30, 35 and 45 ppt, yield of *C. vulgaris* was similar at 1x10^6 cells.ml^{-1}(Fig. 3).

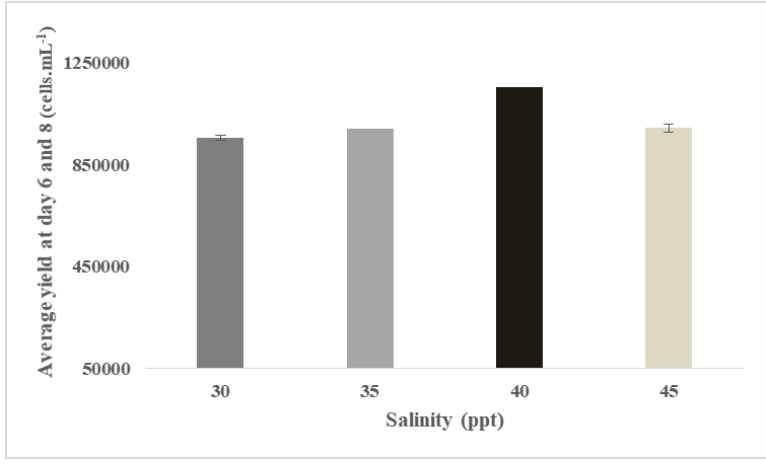

Figure 3. Yield of *C. vulgaris* (mean ± SE) at different salinity during stationary phase of growth

Microalgae Dry Weight

Similar dry weight of *C. vulgaris* was obtained at all salinity tested. Dry weight ranged from 0.03-0,035 g.L^{-1} when cultured at lower salinity (30 and 35 ppt) and 0.04-0.045 g.L^{-1} at higher salinity (40 and 45 ppt) (Fig. 4)

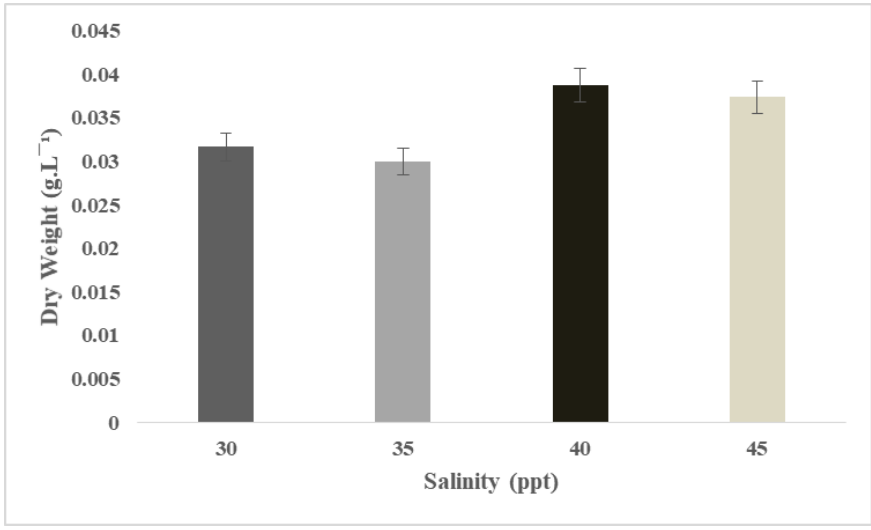

Figure 4. Dry weight (mean ± SE) of *C. vulgaris* at different salinity

Biomass Productivity

Biomass productivity of *C. vulgaris* was similar (p=0.21>0.05) across all salinities tested, ranged from 0.02-0.03 $g.L^{-1}.day^{-1}$ (Fig. 5)

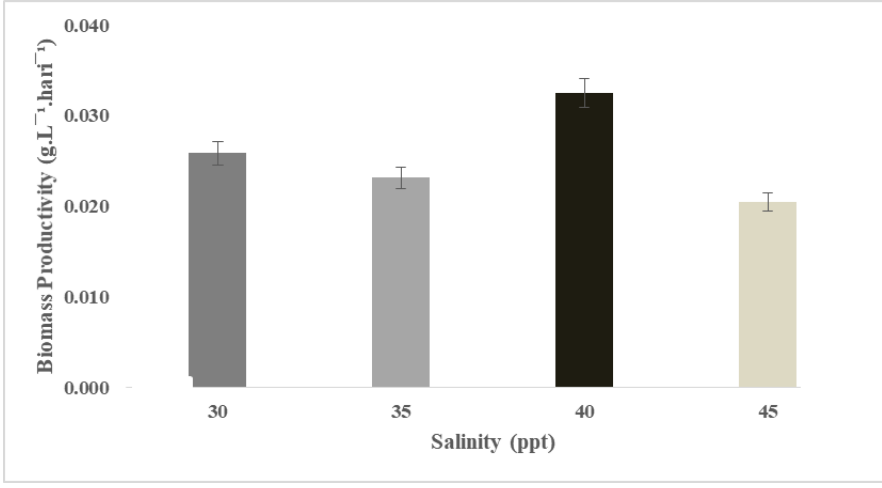

Figure 5. Biomass productivity (mean ± SE) of *C. vulgaris* at different salinity

Discussion

Growth of *C. vulgaris* in this study was significantly affected by salinity with better growth at high (40 and 45 ppt) compared to low (30 and 35) salinities tested. Iba (2016) stated that the growth and nutrients composition of *C. vulgaris* may vary, depending on nutrient in culture media and environmental conditions such as light intensity, temperature and salinity. N and P content in the culture media in this study was considerably higher with 1500 ppm of N and 64 ppm of P in 1 L of culture media) compared to a common culture media such as f/2 that only contains 75 ppm of N and 5 ppm of P in 1 L culture media (FAO, 1996). Goa et al. (2019) found that concentration under 10 % of water hyacinth liquid organic fertilizer produced a better growth of *C. vulgaris*

compared to concentration above it. This may due to the appropriate content of N and P in the culture media that may best utilized by *C. vulgaris*. Triastuti et al. (2011) stated that nitrogen and phosphorus are nutrients needed by microalgae in optimum quantities for growth and development. Nitrogen is an element needed for chlorophyll formation. Phosphorus plays a role in the transfer of energy in cells in the form of ATP. Moreover, Hakalin et al. (2014) stated that one important factor in the successful production of microalgae biomass is the availability of appropriate nutrient in the culture media. These nutrients are very important for cell division and cell metabolic processes. Therefore, if nutrients such as nitrogen (N) and phosphorus (P) run out or are limited in the media, it will subsequently decreased the rate of microalgae reproduction or division thus growth of microalgae.

Cultivation of *C. vulgaris* was in a lag phase (adaptation) from the beginning until the 2nd day of culture period at all salinities tested (Fig. 1). This may have occurred because the cells were cultured in f/2 media during adaptation period at similar salinity prior the culture experiment. Therefore the cells need to adapt with the organic fertlizer culture media. Lag phase in *Chorella*sp. or other microalgae species culture is common and usually occurred on days 1-3 during culture period as a way to adapt to the new environment (Ambar, 2009). During lag phase, the cells divide slowly thus the the number of cells does not increase much (Prayitno, 2015). Moreover, the ability of each microalgae to adapt varies depending on the species and change in salinity of the original habitat or cultue media thus affect their productivity (Widianingsih, 2011; Rudiyanti, 2011).

Considering that *C. vulgaris* culture in this study was supplied with the same amount of nutrients particularly of those N and P, it is suggested that salinity was the main factor that affected growth. Wood et al. (2005) stated that the rate of growth of microalgae cells in a culture is proportional to the availability of nutrients in additon to salinity. Salinity affects the rate of photosynthesis and the osmotic pressure of culture media which is a balance between the cell and its environment (Zainuddin et al., 2017). Also, Ratri and Hermana (2013) stated that if the media salinity is appropriate, there will be a balance of osmotic pressure between microorganism cells and fertilizer media so that growth can be optimal. Increasing cell density occurred significantly during exponential phase of growth and usually is characterized by changes in the color of the culture media. Before the growth of microalgae cells occurred, the culture media color was clear brownish and after the cells underwent the divison and subsequently grow, the culture color changed to greenish. Sintya et al. (2018) stated that during the cultivation of microalgae, physical changes occur in the form of changes in the color of the culture in each phase of growth. In this study, the exponential phase of growth occurred during day 2-4 (Fig. 1) in which at 40 ppt the specific growth rate was higher compared to other salinities. Prayitno (2015) stated that during the exponential growth phase, cells divide rapidly when the enzymes and metabolites needed for cell division are available. On the 10th day *C. vulgaris* experienced a decrease in population where *C. vulgaris* could not maintain its cell density.

Despite the culture media, salinity has been known to affect the growth of microalgae (Iba et al., 2018; Sukmawan et al., 2012) and reconfimerd by this present study. *C. vulgaris* cell growth increased to reach the highest cell density on day 8 of culture (the final stationary phase) when cultured in 40 ppt of liquid organic fertilizer (Fig. 1 and 3). Similar result was found by Waney (2015) when *Chorella* sp. was cultured with different doses of liquid water hyacinth organic fertilizer, they grew better in the salinity range of 30-41 ppt. Djunaedi et al. (2017) found that 35 ppt salinity was an optimal salinity for the growth of *C. vulgaris* when cultured in Walne media. Whereas Nurdiana et al. (2017) found that *Chorella sp*. grew optimally at 30 ppt salinity when cultured in Conway media and vitamin B12. According to Chalid et al. (2010), the marine microalgae species of *Chorella sp*. can tolerate salinity in their environments from 0 - 70 ppt.

Higher growth and cells density of *C. vulgaris* at 40 ppt was followed by higher dry weight (DW) thus biomass productivity

compred to other salinity tested (Fig. 4 and 5). Asia et al. (2018) stated that salinity affects the rate of cell division which will further affect the biomass and productivity of microalgae in culture condition. Moreover, Abdurrachman et al. (2013) found that the increase in biomass is due to the presence of CO_2 gas, which is then absorbed by microalgae and used to carry out photosynthesis, where the the end product of photosynthetic process is carbohydrate which is the main source of biomass.

Light intensity is another factor that affect the growth of *C. vulgaris*. According to Ngagkham et al. (2012) that *C. vulgaris* has the ability to use organic substances in both light and dark conditions. The light intensity used during the study was 540 lux that was equal to 10 µmol photons $m^{-2}.s^{-1}$ PAR with 12:12 ligt: dark period and gentle mixing once a day. According to Sudhakar et al. (2011) that the light needed by microalgae in the process of photosynthesis has a certain limit or range, in general, greater light intensity is more effective for photosynthesis, but at very high light levels can reduce the speed of the process. Boroh et al. (2019) stated that in the culture room the light intensity may rangedfrom 500 - 5000 lux.However, the light intensity used in this study was below the intensity used in our previous study with other green algae species such as *Tetraselmischui*which was 160-170 PAR (Iba et al., 2018). This is may contributed to the low biomass harvested at the end of culture experiment (Fig.3)

Based on the results of the above studies, the batch culture of *C. vulgaris* on a laboratory scale using 5% water hyacinth liquid organic media was better at 40 ppt salinity compared tp other salinities tested. However, to improve the efficiency and productivity of the culture, further investigation of the lipid, protein and carbohydrate content of *C. vulgaris*is needed before going to mass culture to support various industries such as aquaculture, energy or functional food.

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References

Abdurracham, O., Mutiara, M., Buchori, L. 2013. Pengikatan Karbon Dioksida dengan Mikroalga (*Chorella vulgaris*, *Chlamydomonas* sp., *Spirulina* sp.) Dalam Upaya Untuk Meningkatkan Kemurnian Biogas.*JurnalIlmuTeknologi Kimia danIndustri***, 2(4): 212-216.**

Abyor, N.H dan Ariyanti, D. 2012. Potency of Solar Energy Aplications in Indonesia. *International Journal of Renewable Energy Development***, 1(2): 33-38.**

Adenan, N.S., Yusoff, F. Md., and Shariff, M. 2013. Effect of Salinity and Temperature on the Growth of Diatoms and Green Algae**.** *Journal of Fisheries and Aquatic Science,* **8(2): 397-404.**

Ambar, D.P. 2009. Optimasi Pengembangan Media Untuk Pertumbuhan *Chorella* sp. pada Skala Laboratorium. Program Studi Ilmu dan Teknologi Kelautan. *Fakultas Perikanan dan Ilmu Kelautan. Institut Pertanian Bogor*..108 hal.

Amini, S dan Susilowati, R. 2010. Produksi Biodiesel dari Mikroalga *Botryococcus braunii*.*Squalen*, 5(1):23-32.

Ansoruddin. 2014. Kajian Pemberian Dregs (Ampas Tahu) dan Pupuk Nitrogen Teprhadap Pertumbuhan dan Produksi Tanaman Kailan (*Brasisca Olearaceae* Var. Achephala). *Jurnal Penelitian Pertanian Bernas*, 11(1):1-12.

Asia, N., Idris, M., Rahman, A., Kurnia, A., dan Effendy, I.J. 2018. Identifikasi Jenis dan Kepadatan Bentik Mikroalga dari *Enhalus acoroides* dan *Gracillaria arcuata* yang Dikultur pada Bak Sistem IMTA (Integrated Multi Trophic Aquaculture). *Media Akuatika*, 3(1): 581-589.

BBPBAP. 2015. Laporan Penelitian Tahunan Laboratorium Pakan Hidup. *Jepara. Jawa Tengah.*

Chalid, S.Y., Amini, S., dan Lestari, S.D. 2010. Kultivasi *Chlorella* sp. Pada Media Tumbuh yang Diperkaya dengan Pupuk Anorganik dan Soil Extract. *UIN Syarif Hidayatullah Press*. Jakarta, hal298-304.

Chien, Y. H. 1992. Water Quality Requirement and Management for Marine Shrimp

Culture. Review. *Water Quality Management*, pp 144-150.

Chrismadha, T. 1993. Growth and Lipid Production of *Phaedodactylum tricornutum* Bohlin in a Tubular-Photobioreactor. *Thesis. Perth: Murdoch University*, 211 p.

Daliry, S., Hallajisani, A., Mohammadi, J.R., Nouri, H., and Golzary, A. 2017. Investigation of Optimal Condition for *Chlorella vulgaris* Microalgae Growth. *Global J. Environ. Sci. Manage*, 3(2): 217-230.

Dang, N.M., Lee, K. 2018. Utilization of Organic Liquid Fertilizer in Microalgae Cultivation for Biodiesel Production**.** *Biotechnol BioprocessingEnginering*, 23: 405. https://link.springer.com/article/10.1007/s12257-018-0081-3

Djunaedi, A., Sunaryo., Adi, C.S., dan Santosa, A. 2017. Kandungan Pigmen Fikobiliprotein dan Biomassa Mikroalga *Chlorella vulgaris* pada media dengan Salinitas Berbeda. *Jurnal Kelautan Tropis*, 20(2):112-116.

FAO.1996. Manual on the Production and Use of Live Food for Aquaculture. FAO. Rome

Goa, S., Iba, W., Indrayani. 2019. Pengaruh Dosis Pupuk Organik Cair Eceng Gondok (*EichhorniaCrassipes*) yang Berbeda Terhadap Pertumbuhandan Kepadatan Sel Mikroalga *Chlorella vulgaris. Media Akuatika*, 4(2): 68-76.

Hadiyanto dan Azim, M. 2012. Mikroalga Sumber Pangan dan Energi Masa Depan.C-Biore (Center of Biomass and Renewable Energy). *UPT UNDIP Press Semarang*. Semarang,1-115 hal.

Hakalin, N.L.S., Paz, A.P., Aranda, D.A.G., Moraes, L.M.P. 2014. Enhancement of Cell Growth and Lipid Content of a Freshwater Microalga *Scenedesmus* sp. by Optimizing Nitrogen, Phosphorus and Vitamin Concentrations for Biodiesel Production. *Natural Science*, 6(1): 1044-1054.

Iba, W., Rice, M.A., Maranda, L., and Wikfors, G.H. 2018. Growth Characteristics of Newly Isolated Indonesian Microalgae Under Different Salinity. *Indonesian Aquaculture Journal*, 13(2): 71-81.

Iba, W. 2016. The Potential of Indonesia Microalgae to Support White Shrimp (*Litopenaeus vannamei*) Aquaculture. *Disertation University of Rhode Island*, pp. 176.

John, M. 2016. Production of Organic Compost from Water Hyacinth (*Eichhorniacrassipes* [Mart.] Solms) in the Lake Victoria Basin: A Lake Victoria Research Initiative (VicRes).*Research & Reviews: Journal of Agriculture and Allied Sciences*, 5(2): 50-57

Kawaroe, M., T., Prartono, A., Sunuddin, D. W., Sari, D., dan Augustine. 2010. Mikroalga: Potensi dan Pemanfaatannya untuk Produksi Bio Bahan Bakar. *IPB Press. Bogor.*

Kawaroe, M., Prartono, T., Rachmat, A., Wulan, D.S., dan Agustine, D. 2012. Pertumbuhan Spesifik dan Kandungan Asam Lemak pada Mikroalga *Spirulina plantensis, Isochrysis* sp. dan *Porphyridium cruentum. Jurnal Ilmu Kelautan*, 17(3): 125-131.

Maesaroh, S.L. 2016. Pertumbuhan dan Kualitas Biomassa *Spirulina platensis* yang di Produksi Pada Media Zarouk Modifikasi. *Skripsi. Universitas Islam Negeri Syarif Hidayatullah, Jakarta.*

Moia, R. A., Pandiangana, D., Siahaana, P., dan Tangapo, M.A. 2015. Pengujian Pupuk Organik Cair dari Eceng Gondok (*Eichhornia crassipes*) Terhadap Pertumbuhan Tanaman Sawi (*Brassica juncea*). *Jurnal MIPA UNSRAT Online*, 4 (1) : 15-19.

Najmuddin, F.Z. 2011. Peningkatan Produksi Biomassa *Chorella vulgaris* dengan Perlakuan Mikrofiltrasi pada Sirkulasi Aliran Medium Kultur Sebagai Bahan Baku. *Skripsi. Fakultas Teknik. Departemen Teknik Kimia. Depok.*

Nurdiana, S., Sarwono., dan Nikhlani, A. 2017. Kepadatan Sel *Chorella* sp. yang Dikultur dengan Periodisitas Cahaya Berbeda. *Jurnal Aquawarman*, 3(2):35-41.

Oktovianus, S.G. 2018. Mikroalga: Sumber Energi Terbarukan Masa Depan. *Jurnal Kelautan*, 11(1): 95-103.

Osoro, N., Muoma, J.O., Amoding, A., Mukaminega, D., Muthini, M., Omboro, O., Maingi, J.M. 2014. Effects of Water Hyacinth (*Eichhorniacrassipes* [mart.]solms) Compost on Growth and Yield Parameters of Maize (Zea mays). *Current Journal of Applied Science and Technology* 4(4):617-633

Pratama, I. 2011. Pengaruh Metode Pemanenan Mikroalga Terhadap Biomassa dan Kandungan Esensial *Chorella vulgaris.Skripsi. Depok. Indonesia*.

Prayitno, J. 2015. Pola Pertumbuhan dan Pemanenan Biomassa dalam Fotobioreaktor Mikroalga untuk Penangkapan Karbon. *Jurnal Teknologi Lingkungan*, 17(1): 45-52.

Ratri, D.M.I dan Hermana, J. 2013. Pengaruh Konsentrasi Bahan Organik, Salinitas, dan pH Terhadap Laju Pertumbuhan Alga. *Seminar Nasional PascasarjanaXIII*. 1-6 hal.

Ravi, O.P.K., Kumar, S., Kumari, S., Kolhe, S.S., Singh, A.K. 2019. A Brief Review on *Eichhornia* Extract as Liquid Fertilizers for Aquaculture Pond. *Int.J.Curr.Microbiol.App.Sci*, 8(3): 1044-1051

Ru'yatin., Suci, I.R., dan Ali, L. 2015. Pertumbuhan *Tetraselmis* dan *Nannochloropsis* pada Skala Laboratorium. *Jurnal Pros Sem Nas Masy Biodiv Indon*, 1(2): 296-299.

Rudiyanti, S. 2011. Pertumbuhan *Skeletonema costatum* Pada Berbagai Tingkat Salinitas Media. *Jurnal Saintek Perikanan*, 6(2): 69-76.

Sanni, K. O., Adesina, J. M. 2011. Utilization of water hyacinth (*Eichhorniacrassipies*Mart Solms) as liquid fertilizer on the growth and yield of fluted pumpkin (*Telfairiaoccidentalis*).*South Asian Journal of Experimental Biology*, 2(1): 33-37

Setyaningsih, F.P., Retnaningsih, T.S., dan Izzati, M. 2013. Pertumbuhan *Chlorella Vulgaris* Beijerinck dalam Medium yang Mengandung Logam Berat Cd dan Pb Skala Laboratorium. *Prosiding Seminar Nasional Biologi*, 104-116 hal.

Setyaningsih, F.P., Retnaningsih, T.S., dan Izzati, M. 2015. Potensi *Chlorella vulgaris* Beijerinck Dalam Remediasi Logam Berat Cd Dan Pb Skala Laboratorium**.** *BIOMA*, 16(2): 102-113.

Sintya, M.D., Samiaji, J., dan Nurrachmi, I. 2018. Pengaruh Perbedaan Salinitas Terhadap Pertumbuhan Biomassa Kultivasi Mikroalga *Spirulina platensis* Pada Skala Semi Outdoor. *Skripsi. Universitas Riau, Indonesia.*

Sipauba-Tavares, L.H., Maria, A.D.L.S., Almeida, F.B.M., and Scardoeli, B.T. 2017. Development of Low-cost Culture Media for *Ankistrodesmus gracilis* based on Inorganic Fertilizer and Macrophyte. *Acta Limnologica Brasiliensia*, 29(5): 159-179.

Sudhakar, K., Suresh, S., dan Premalatha, M. 2011. An Overview of CO_2 Mitigation Using Algae Cultivation Technology. *International Journal of Chemical Research*, 3: 110-117.

Sukmawan, A. M., Antara, S. N., dan Arnata, W. I. 2012. Optimization Salinity and Initial pH On The Biomass Production of *Nannochloropsis* sp. K-4. *Jurnal Rekayasa dan Manajemen Agroindustri. Fakultas Teknologi Pertanian, Universitas Udayana Bali. Indonesia.*

Suminto. 2009. Penggunaan Jenis Media Kultur Teknis Terhadap Produksi dan Kandungan Nutrisi Sel *Spirulina platensis.Jurnal Saintek Perikanan*, 4(2): 53-61.

Syawal, Y. 2010, Pertumbuhan Tanaman Lidah Buaya dan Gulma yang diaplikasi Bokhasi Enceng Gondok dan Kiambang serta Pupuk Urea**.** *Jurnal Agrivigor*, 10(1):108-116**.**

Triastuti, R.J.A., Mubarak, S., dan Prabandari, L. 2011. Pengaruh Penambahan Pupuk Bintil Akar Kacang Tanah Sebagai Sumber Nitrogen dan Fosfor Terhadap Populasi *Chorella* sp**.** *Jurnal Ilmiah Perikanan dan Kelautan*, 3(2): 157-163.

Umbu, I.J. 2014. Kandungan Total Lipid *Chorella vulgaris* yang dikultur dalam media yang Diinjeksi Co_2**.** *Journal of Marine Research*, 3(4): 578-585.

Waney, L., Konio, Y.., dan Muharam, A. 2015. Pengaruh Penambahan Ekstrak Eceng Gondok (Eichhornia crassipes) Terhadap Laju Pertumbuhan Pakan Alami Chorella sp**.** *Universitas Negeri Gorontalo***, Indonesia.**

Widianingsih., R. Hartati., E.H. Endrawati., M. Hilal. 2011. Kajian Kadar Total Lipid dan Kepadatan *Nitzschia* sp. yang Dikultur dengan Salinitas Berbeda. Undip E-Journal 4030-8655-1.

Zainuddin, M., Hamid, N., Mudiarti, L., Kursistyanto, N., dan Aryono, B. 2017. Pengaruh Media Hiposalin dan Hipersalin Terhadap Respon Pertumbuhan dan Biopigmen*Dunaliella salina. Jurnal Enggano*, 2(1): 46-57.