Journal of Aquaculture and Fish Health Vol. 11(1) - February 2022
DOI : 10.20473/jafh.v11i1.21345

The Performance of Microalgae (*Nannochloropsis* sp., *Tetraselmis* sp. and *Dunaliella* sp.) on White Shrimp (*Litopenaeus vannamei*) Wastewater Cultivation Media

Muhammad Browijoyo Santanumurti[1], Santrika Khanza[2], Zaenal Abidin[3], Berta Putri[2] and Siti Hudaidah[2]*

[1]Department of Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Jl. Mulyorejo, Mulyorejo, Surabaya 60115, Indonesia
[2]Department of Fisheries and Marine Science, Faculty of Agriculture, University of Lampung, Jl. Prof. Sumantri Brojonegoro, Bandar Lampung, Lampung 35141, Indonesia
[3]Department of Aquaculture, National Taiwan Ocean University, No. 62, Pei-Ning Road, Keelung 20224, Taiwan, ROC

*Correspondence :
idahasan64@gmail.com

Received : 2020-08-11
Accepted : 2021-08-02

Keywords :
Microalgae, Wastewater, White shrimp

Abstract

Microalgae have an important role in supporting the development of aquaculture because they can be used as natural feed. However, its culture requires an expensive cost because of the nutrient media. To reduce the cost, the media can be replaced by using wastewater from white shrimp (*Litopenaeus vannamei*) culture. This research was aimed to find out the performance of microalgae (*Nannochloropsis* sp., *Tetraselmis* sp. and *Dunaliella* sp.) cultured on white shrimp wastewater. The performance was measured by the growth, density, and ability to reduce nitrate and phosphate. The experimental design used in this study was a Completely Randomized Design with three treatments and three replications. The treatments were A (*Nannochloropsis* sp. cultured in white shrimp wastewater), B (*Tetraselmis* sp. cultured in white shrimp wastewater), and C (*Dunaliella* sp. cultured in white shrimp wastewater). The density population of *Nannochloropsis* sp., *Tetraselmis* sp. and *Dunaliella* sp. were tested by ANOVA. ANOVA was used to assess the density population of Nannochloropsis sp., Tetraselmis sp., and Dunaliella sp., which was then followed by Duncan's test. The results showed that wastewater from white shrimp aquaculture could be used as a medium culture for *Nannochloropsis* sp., *Tetraselmis* sp. and *Dunaliella* sp. It also maintains good water parameter quality in media. *Nannochloropsis* sp. was the microalgae that produced the highest density of 34.5 x 10^4 ind/mL when cultured on wastewater from white shrimp culture. *Nannochloropsis* sp. may also reduce nitrate and phosphate content by up to 76 and 61.37 percent, respectively.

INTRODUCTION

Microalgae play an important role in supporting the development of aquaculture. That is because microalgae can be used as natural feed for larvae due to their high nutrient. Microalgae have protein and fat values reaching 71% and

77% (Barkia *et al*., 2019). Microalgae also contain bioactive compounds, minerals, vitamins, sterols, essential amino acids, as well as pigments (chlorophyll, carotenoids, and phycobiliprotein) (Yadav *et al*., 2020). In addition to the high nutritional, the use of microalgae as a feed meets the criteria, such as easy to obtain, economical value, and non-toxic (Daniel *et al*., 2016; Han *et al*., 2019). Therefore, much research and application are related to microalgae culture to support the development of aquaculture.

Microalgae culture is applied by using synthesis fertilizers such as Walne and Conway. Unfortunately, the use of such fertilizers requires quite high fees and limited availability (Indriana *et al*., 2020). Walne also contains boron which can cause the loss of pigment (Boroh *et al*., 2019). Therefore, there needs to be a culture media other than Walne and Conway as an alternative to reduce costs in the production of microalgae.

One alternative that can be done in microalgae culture activity is by using the wastewater white shrimp (*Litopenaeus vannamei*). In the cultivation of white shrimp, about 25-30% nitrogen and phosphorus in the feed is utilized by shrimp, and the rest is wasted in the water as organic material (Syah *et al*., 2014). Apart from the uneaten feed, organic material in shrimp ponds is also caused by feces that settles at the bottom (Manan *et al*., 2017). Such organic material rot will accumulate at the base of the water and affect the quality of the aquatic environment so that the water change is needed in the pond (Chen *et al*., 2018). The process of changing the water in the pond is done by disposal through the top of the pond, the result is also called the wastewater shrimp (Harianja *et al*., 2018). Water change activities in the pond impact the environment and ecosystem surrounding the form of organic material content, so that management is needed to keep the cultivation activities ongoing and sustainable (Saiya and Katoppo, 2015).

The utilization of wastewater from white shrimp culture that is rich in nutrients becomes one of the alternatives as a culture media of microalgae and wastewater management. Nitrates (N) and phosphate (P) are nutrients that are widely contained in the wastewater from white shrimp culture, where the production is influenced by the artificial feed given during culture activity (Agis and Wahyu, 2015).

The percentage of N and P released into the environment per ton of fish in the intensive aquaculture system ranged from 81.5% and 85.7% and which stored as biomass fish only ranged from 18.5% and 14.3% (Islam, 2005). Microalgae require nitrogen and phosphate to grow (Amini *et al*., 2019). Previous research has demonstrated the effect of wastewater on microalgae growth. Tangguda and Prasetia (2017) stated that the influence of wastewater from shrimp ponds produced cell density of *Chlorella* sp. as much as 86,750 cells/mL. Microalgae that grow in the wastewater of shrimp culture is not only increasing the biomass but also plays an important role in the decomposition of aquaculture waste thereby lowering the burden of contamination (Zheng *et al*., 2017). This study aimed to determine the performance of microalgae (*Nannochloropsis* sp., *Tetraselmis* sp. and *Dunaliella* sp.) on wastewater from white shrimp culture. The performance was not only about the microalgae density, but also the influence of microalgae on water quality parameters, especially nitrates and phosphate in the water.

METHODOLOGY

Place and Time

The study was conducted in April-May 2019 in Fisheries Laboratory, Department of Fisheries and Marine, Faculty of Agriculture, University of Lampung.

Research Materials

Nannochloropsis sp. and *Tetraselmis* sp., were obtained from the Balai Besar Perikanan Budidaya Laut (BBPBL)

Lampung while *Dunaliella* sp. was received from the Marine Fisheries Center or Balai Besar Perikanan Budidaya Air Payau (BBPBAP) Jepara. Wastewater in this study is used from the white shrimp pond in Lemong district, West Coast District, Lampung.

Research Design

The experimental study used in this study was Complete Random Design. The treatment used in this study was treatment A (*Nannochloropsis* sp. cultured in white shrimp wastewater), B (*Tetraselmis* sp. cultured in white shrimp wastewater), and C (*Dunaliella* sp. cultured in white shrimp wastewater). The treatments in this study consisted of three replications.

Work Procedure

Microalgae Inoculant Preparation

Nannochloropsis sp., *Tetraselmis* sp. and *Dunaliella* sp. Preparation was started by preparing a 3 L glass jar for each and filled with 2 L of seawater. The 2 mL of Conway fertilizer was added for *Nannochloropsis* sp. and *Tetraselmis* sp. while 2 mL of Walne was added for *Dunaliella* sp. The use of different nutrients was expected to get optimum results for algae culture. Then the 200-400 mL inoculant was added (10-20% of the volume of water) and added aeration, closed, and cultured for 5 days so that the microalgae were ready to be used.

Media Preparation

The media preparation was done according to the previous study (Utomo *et al*., 2005). Microalgae culture container with a volume of 500 mL filled with wastewater from white shrimp culture in Lemong district, West Coast District, Lampung that had been sterilized. Containers were installed with TL lamp 36 watts with an average intensity of 3500 lux, as a light source during microalgae culture. Culture media were aerated and added with *Nannochloropsis* sp., *Tetraselmis* sp. and *Dunaliella* sp. from the inoculant preparation process with a density of 10^4 individual/mL.

Microalgae Density Calculation

The main parameter in the study was the population growth and density of microalgae (*Tetraselmis* sp., *Nannochloropsis* sp. and *Dunaliella* sp.). Density calculation was conducted by using a Neubauer Chamber. Calculation of microalgae density was carried out after 24 hours of initial dispersion every day for 15 days. The calculation was done three times in one day at 08.00 am by setting 1 mL of the sample on the part of the Neubauer Chamber to the full trench after it was observed under the binocular microscope with a magnification of 10x and calculated by the formula (Boroh *et al*., 2019):

$$k = n \times p \times 2500$$

Where:

k = cell density (individuals/mL)
n = total cell numbers
p = dilution level
2500 = counting chamber

Water Quality Measurement

Measurement of nitrate levels was carried out using the method of testing nitrate levels in water using a brucine sulfate spectrophotometer (SNI 06-2480-1991) (Nandiyanto and Haristiani, 2017). This method was used to determine the number of nitrate levels by brucine sulfate using a spectrophotometer at a wavelength of 410 nm. Nitrate analysis was performed at baseline and end throughout the study. Measurement of phosphate levels was carried out using the method of how to test for phosphate levels using a spectrophotometer using ascorbic acid (SNI 06-6989.31-2005) (Dewi *et al*., 2019).

This method was used to determine the number of phosphate levels by ascorbic acid by using a spectrophotometer at a wavelength of 880 nm. Phosphate and nitrate analyses were carried out at baseline and end during the study. Other water quality parameters observed were pH, salinity, temperature,

and light intensity which were measured every 24 hours according to previous research (Sulistiowati *et al*., 2016).

Data Analysis

The peak density data of microalgae were tested for normality and homogeneity with a time of 1-day retrieval for 15 days. If the data was significant (> 0.05), ANOVA (Analysis of Variance) would be conducted at a 95% confidence level. Once the data was significant, it was followed by a double comparative test of Duncan (Dunn's Multiple Range Test) with a confidence rate of 95% to determine the significant difference between treatments. Water quality data was analyzed descriptively and presented in a table form.

RESULTS AND DISCUSSION

Microalgae Performance

Microalgae performance could be seen from the population growth and density value of each microalga (*Nannochloropsis* sp., *Tetraselmis* sp. and *Dunaliella* sp. using wastewater from white shrimp culture as a culture medium. The population growth value for 15 days showed in Figure 1.

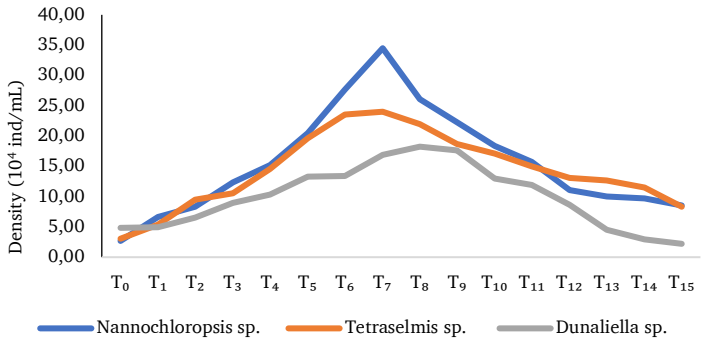

Figure 1. Daily population growth of microalgae (*Nannochloropsis* sp., *Tetraselmis* sp. and *Dunaliella* sp.) during the 15 days of the study.

Population growth in treatment A underwent an adaptation phase on day 0 to day 4 with a density of 15.23 x 10^4 ind/mL while the exponential phase occurred on day 5 to day 7 with a density of 34.5 x 10^4 ind/mL. The stationary phase occurred on the 8th day with a density of 26.06 x 10^4 ind/mL and the death phase decreased the growth rate of *Nannochloropsis* sp. on day 9 to day 15 with a density of 9 x 10^4 ind/mL.

The growth of the microalgae population in treatment B underwent an adaptation phase on day 0 to day 3 with a density of 10.58 x 10^4 ind/mL. The exponential phase occurred from day 4 to day 7 at 24.01 x 10^4 ind/mL. Then the stationary phase occurred on the 8th day of 21.96 x 10^4 ind/mL and the death phase occurred on the 11th day to the 15th day with a density of 8.33 x 10^4 ind/mL.

The growth of the microalgae population in treatment C underwent an adaptation phase on day 0 to day 2 with a density of 6.48 x 10^4 ind/mL while the exponential phase occurred on day 3 to day 8 with a density of 18.24 x 10^4 ind/mL. Then the stationary phase occurred on the 9th day at 17.6 x 10^4 ind/mL and the death phase occurred on the 10th to 15th day with a density of 2.21 x 10^4 ind/mL. The density of *Nannochloropsis* sp., *Tetraselmis* sp. and *Dunaliella* sp. at the peak phase was presented in Figure 2.

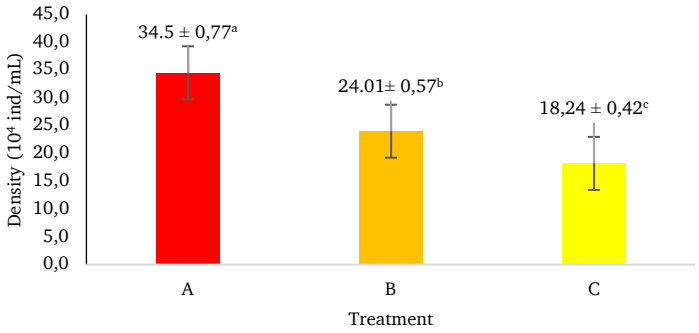

Figure 2. Population density of the microalgae at the peak phase. Different superscripts showed significant differences.

The population of *Nannochloropsis* sp. showed the highest density than others. The peak phase of its population generated a density of 34.5 x 10^4 ind/mL while on *Tetraselmis* sp. and *Dunaliella* sp. produced a density of 24.01 x 10^4 ind/mL and 18.24 x 10^4 ind/mL. The results of this research indicated that all microalgae could be cultured by using wastewater of white shrimp culture by research (Tangguda and Prasetia, 2017). This was because microalgae could absorb inorganic materials in wastewater aquaculture and used them to grow (Ansari *et al*., 2017)

Based on peak growth and density, *Nannochloropsis* sp. showed the best performance. According to Diharmi (2001), *Nannochloropsis* sp. was one of the most efficient algae in utilizing light and oxygen energy for the photosynthesis process, where the microalgae were able to breed using only sunlight, oxygen, and seawater. The cell size of microalgae also affected the absorption of nutrients that existed in the culture media. *Nannochloropsis* sp. had a diameter size of 2-4 µm, while *Tetraselmis* sp. was 7-12 µm and *Dunaliella* sp. was1-15 µm (Ben-Amotz *et al*., 2009).

A previous study indicated that larger size would compete intensely than smaller size (Ciotti *et al*., 2002). Each microalgae cell had a different nutrient absorption force, depending on the functional group content of the cell wall and the ion exchange occurring on its cell surface. Also, the cell surface area of each microalgae type affected the absorption rate of nutrients (Fachrullah, 2011). *Nannochloropsis*'s cell wall contains algaenans which was expected to block enzyme access (Scholz *et al*., 2014). *Tetraselmis*' cell wall was known as theca which contained Kdo, 50MeKdo, DHA, galacturonic acid, galactose, gulose, and arabinose (Becker *et al*., 2002). *Dunaliella* was lack of cell wall due to inhibition of dichlobenil (Felix *et al*., 1988).

The lowest microalgae density occurred in the culture of *Dunaliella* sp. The smaller the size of the cell, the larger the surface area so that the nutrient entry into the cell tissue was faster (Musa *et al*., 2013). Therefore, the abundance of microalgae cells among the treatment of one of them depended on the ability of microalgae in absorbing nutrients contained in the maintenance media.

Water Quality Parameter

Nitrate and phosphate parameters were measured at the start and end of the study. The results of measurements of nitrate and phosphate levels were presented in Table 1.

Table 1. Nitrate and phosphate content during the study.

Treatment	Nitrate (NO_3) (mg/L)		Reduction (%)	Phosphate (PO_4) (mg/L)		Reduction (%)	N:P	
	Initial	Final		Initial	Final		Initial	Final
A	5.59	1.31	76,5	6.55	2.53	61.37	1:1.2	1:1.9
B	5.19	8.69	-67,4	6.53	3.35	51.30	1:1.3	1:2.6
C	5.45	2.54	53.40	6.52	3.07	48.77	1:1.2	1:1.2

Total nitrate (NO_3) results showed that treatment A decreased by 4.28 mg/L with a decreasing percentage of 76.56%. In treatment B, there was an increase of 3.5 mg/L with a percentage of an increase of 67.53% and in treatment C there was a decrease of 2.54 mg/L with a percentage of a decrease of 53.40%. The phosphate value (PO_4) results showed that treatment A decreased by 4.02 mg/L with a decreasing percentage of 61.37%. In treatment, B decreased by 3.45 mg/L with a decreasing percentage of 51.30%, and in treatment C, decreased by 3.18 mg/L with a percentage decrease of 48.77%.

Nannochloropsis sp. and *Dunaliella* sp. could decrease nitrate levels at the end of the study. The reduction in nitrate levels was due to microalgae consuming N as its growth nutrient, where nitrate played a role in the process of forming amino acids, fats, and vegetative cells. Otherwise *Tetraselmis* sp. increased the nitrate levels at the end of the study. It was occurred probably due to the *Tetraselmis* sp. could not utilize the nutrients contained in wastewater from white shrimp culture. According to the previous study (Hastuti, 2001), the higher the dose of waste content, the lower the effectiveness of nutrient utilization by microalgae. According to Han *et al.* (2019), another factor that caused a higher nitrate content was probably due to the high density of microalgae in treatment B so that at the end of the study the microalgae experienced death and then decomposed into nitrates.

The important factor that influenced the growth of microalgae during culture was water quality. The following results of water quality measurements during the study can be seen in Table 2.

Table 2. Water quality parameters during the study.

Parameter	Treatment			Optimum
	A	B	C	
Temperature (°C)	23-25	23-25	23-25	23-25[(1)] and 21-30[(2)]
pH	8,5-8,9	8,6-8,9	8,2-8,6	8-9,5[(3)]
Salinity (ppt)	25-30	25-32	25-35	25-35[(3)] and 20-35[(4)]
Light intensity (lux)	2427-4240	2240-4503	2443-4190	1000-10000[(5)] and 2000-8000[(6)]

Source :1. Fachrullah (2011) (optimum temperature for *Nannochloropsis* sp. and *Tetraselmis* sp.)
2. Yudha (2008) (optimum temperature for *Dunaliella* sp.)
3. Barsanti and Gualtieri (2005)
4. Chen *et al.* (2011) (optimum salinity fro *Dunaliella* sp.)
5. Balai Budidaya Laut (2002) (optimum light intensity for *Nannochloropsis* sp. and *Tetraselmis* sp.)
6. Rostini (2007) (optimum light intensity for *Dunaliella* sp.)

The results of *Nannochloropsis* sp. during the study were 23-25°C, pH of 8.5-8.9, salinity ranging from 25-30 ppt, and light intensity of 2427-4240 lux. The results of *Tetraselmis* sp. during the study were 23-25°C, pH of 8.6-8.9, salinity ranging from 25-32 ppt, and light intensity of 2240-4503 lux. The results of *Dunaliella* sp. during the study were 23-25°C, pH of 8.2-8.6, salinity ranging from 25-35 ppt,

and light intensity of 2443-4190 lux. These results indicated that all microalgae in this study could maintain water quality according to optimum standards.

CONCLUSION

Wastewater from white shrimp aquaculture could be utilized as media culture for microalgae *Nannochloropsis* sp., *Tetraselmis* sp. and *Dunaliella* sp. *Nannochloropsis* sp. showed the best performance because it was able to produce the highest density and mostly reduce the content of nitrates and phosphate in the culture media.

ACKNOWLEDGMENT

We thank all parties who have aided in the completion of this study, especially Universitas Airlangga, Surabaya, and Universitas Lampung, Lampung. We also thank Santrika Khanza for her cooperation in completing this study.

REFERENCES

Agis, R. and Wahyu, S., 2015. *Budidaya ikan kerapu macan*. WWF Indonesia.

Amini, M., Khoei, Z.A. and Erfanifar, E., 2019. Nitrate (NO_3-) and phosphate (PO_4-) removal from aqueous solutions by microalgae *Dunaliella salina*. *Biocatalysis and Agricultural Biotechnology*, *19*, 101097. https://doi.org/10.1016/j.bcab.2019.101097

Ansari, F.A., Singh, P., Guldhe, A. and Bux, F., 2017. Microalgal cultivation using aquaculture wastewater: integrated biomass generation and nutrient remediation. *Algal research*, *21*, pp.169-177. https://doi.org/10.1016/j.algal.2016.11.015

Balai Budidaya Laut, 2002. Budidaya fitoplankton dan zooplankton. Direktorat Jendral Perikanan. Departemen Kelautan dan Perikanan, *9*, pp.7-8.

Barkia, I., Saari, N. and Manning, S.R., 2019. Microalgae for high-value products towards human health and nutrition. *Marine drugs*, *17*(5), p.304. https://doi.org/10.3390/md17050304

Barsanti, L. and Gualtieri, P., 2005. *Algae: Anatomy, biochemistry, and biotechnology*. CRC Press. United States of America. p.301. https://doi.org/10.1201/9780203492598

Becker, B., Melkonian, M. and Kamerling, J.P., 2002. The cell wall (theca) of *Tetraselmis striata* (*Chlorophyta*): macromolecular composition and structural elements of the complex polysaccharides. *Journal of phycology*, *34*(5), pp.779-787. https://doi.org/10.1046/j.1529-8817.1998.340779.x

Ben-Amotz, A., Polle, J.E.W. and Rao, D.V.S., 2009. *The alga Dunaliella: Biodiversity, physiology, genomics and biotechnology*. CRC Press, USA. pp.357-493.

Boroh, R., Litaay, M., Umar, M.R., and Ambeng, A., 2019. Pertumbuhan *Chlorella* sp. pada beberapa kombinasi media kultur. *BIOMA: Jurnal Biologi Makassar*, *4*(2), pp.129-137. https://doi.org/10.20956/bioma.v4i2.6759

Chen, C.Y., Yeh, K.L., Aisyah, R., Lee, D.J. and Chang, J.S., 2011. Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: A critical review. *Bioresource technology*, *102*(1), pp.71-81. https://doi.org/10.1016/j.biortech.2010.06.159

Chen, Z., Ge, H., Chang, Z., Song, X., Zhao, F. and Li, J., 2018. Nitrogen budget in recirculating aquaculture and water exchange systems for culturing *Litopenaeus vannamei*. *Journal of Ocean University of China*, *17*(4), pp.905-912. https://doi.org/10.1007/s11802-018-3584-9

Ciotti, A.M., Lewis, M.R. and Cullen, J.J., 2002. Assessment of the relationships between dominant cell size in natural phytoplankton communities and the spectral shape of the absorption coefficient. *Limnology and Oceanography*, *47*(2),

pp.404-417. https://doi.org/10.4319/lo.2002.47.2.0404

Daniel, N., Sivaramakrishnan, T., Saravanan, K., Shalini, B., Arunjyoti, B., Sankar, R. and Dann Roy, S., 2016. A review on microalgae as potential fish feed ingredient. *Journal of the Andaman Science Association*, *21*(1), pp.140-144. https://www.researchgate.net/publication/311312413_A_Review_on_Microalgae_as_Potential_Fish_Feed_Ingredient

Dewi, N.R., Hadisoebroto, R. and Fachrul, M.F., 2019. Removal of ammonia and phosphate parameters from greywater using *Vetiveria zizanioides* in subsurface-constructed wetland. *Journal of Physics: Conference Series, 1402*(3), 033012. https://doi.org/10.1088/1742-6596%2F1402%2F3%2F033012

Diharmi, A., 2001. *Pengaruh pencahayaan terhadap kandungan pigmen bioaktif mikroalga Spirulina platensis strain local (INK)*. Doctoral dissertation, Institut Pertanian Bogor, Bogor, p.78.

Fachrullah, M.R., 2011. *Laju Pertumbuhan mikroalga penghasil biofuel Jenis Chlorella sp. dan Nannochloropsis sp. yang dikultivasi menggunakan air limbah hasil penambangan timah di Pulau Bangka.* Bachelor Thesis, Institut Pertanian Bogor, Bogor, p.102

Felix, H.R., Chollet, R. and Harr, J., 1988. Use of the cell wall-less alga *Dunaliella bioculata* in herbicide screening tests. *Annals of applied biology*, *113*(1), pp. 55-60. https://doi.org/10.1111/j.1744-7348.1988.tb03281.x

Han, P., Lu, Q., Fan, L. and Zhou, W., 2019. A review on the use of microalgae for sustainable aquaculture. *Applied Sciences*, *9*(11), 2377. https://doi.org/10.3390/app9112377

Harianja, R.S.M., Anita, S. and Mubarak, 2018. Analisis beban pencemaran tambak udang di sekitar Sungai Kembung Kecamatan Bantan Bengkalis. *Dinamika lingkungan Indonesia*, *5*(1), pp.12-19. http://dx.doi.org/10.31258/dli.5.1.p.12-19

Indriana, N., Iba, W., Idris, M., Ruslaini, Abidin, L.O.B. and Aslan, L.O.M., 2020. Pengaruh kosentrasi pupuk organik cair lemna (*Lemna minor*) yang berbeda terhadap pertumbuhan mikroalga *Chlorella vulgaris*. *Media Akuatika*, *5*(1). http://dx.doi.org/10.33772/jma.v5i1.11754

Islam, M.S., 2005. Nitrogen and phosphorus budget in coastal and marine cage aquaculture and impacts of effluent loading on ecosystem: review and analysis towards model development. *Marine pollution bulletin*, *50*(1), pp.48-61. https://doi.org/10.1016/j.marpolbul.2004.08.008

Manan, H., Moh, J.H.Z., Kasan, N.A., Suratman, S. and Ikhwanuddin, M., 2017. Identification of biofloc microscopic composition as the natural bioremediation in zero water exchange of Pacific white shrimp, *Penaeus vannamei*, culture in closed hatchery system. *Applied Water Science*, *7*(5), pp.2437-2446. https://doi.org/10.1007/s13201-016-0421-4

Musa, B., Raya, I. and Dali, S., 2013. *Pengaruh penambahan ion* Cu_2^+ *terhadap laju pertumbuhan fitoplankton Chlorella vulgaris*. Universitas Hasanuddin. Makassar, pp.1-9.

Nandiyanto, A.B.D. and Haristiani, N., 2017 Design of simple water treatment system for cleaning dirty water in the rural area. *IOP Conference Series: Materials Science and Engineering, 180,* 012148. https://doi.org/10.1088/1757-899X/180/1/012148

Rostini, I., 2007. *Kultur fitoplankton (Chlorella sp. dan Tetraselmis chuii) pada skala laboratorium*. Universitas Padjajaran. Jatinagor. 33 p.

Saiya, H.G. and Katoppo, D.R., 2015. Waste management of shrimp farms as starting point to develop integrated farming systems (case study: Kuwaru Coast, Bantul, Yogyakarta, Indonesia). *Journal of Degraded and Mining Lands Management*, *3*(1), pp.423-432. https://doi.org/10.15243/jdmlm.2015.031.423

Scholz, M.J., Weiss, T.L., Jinkerson, R.E., Jing, J., Roth, R., Goodenough, U., Posewitz, M.C. and Gerken, H.G., 2014. Ultrastructure and composition of the *Nannochloropsis gaditana* cell wall. *Eukaryotic cell*, *13*(11), pp.1450-1464. https://doi.org/10.1128/ec.00183-14

Sulistiowati, D., Tanjung, R.H.R. and Lantang, D., 2016. Keragaman dan kelimpahan plankton sebagai bioindikator kualitas lingkungan di perairan Pantai Jayapura. *Jurnal Biologi Papua*, *8*(2), pp.79-96. http://dx.doi.org/10.31957/jbp.56

Syah, R., Makmur and Undu, M.C., 2014. Estimasi beban limbah nutrien pakan dan daya dukung kawasan pesisir untuk tambak udang vaname superintensif. *Jurnal Riset Akuakultur*, *9*(3), pp.439-448. http://dx.doi.org/10.15578/jra.9.3.2014.439-448

Tangguda, S. and Prasetia, I.N.D., 2017. Pengaruh limbah cair tambak udang terhadap kepadatan sel dan laju pertumbuhan spesifik *Chlorella* sp. *Saintek Perikanan : Indonesian Journal of Fisheries Science and Technology*, *14*(2), pp.96-99. https://doi.org/10.14710/ijfst.14.2.96-99

Utomo, N.B.P., Winarti and Erlina, A., 2005. Growth of *Spirulina platensis* Cultured with Inorganic Fertilizer (Urea, TSP and ZA) and Chicken Manure. *Jurnal Akuakultur Indonesia*, *4*(1), pp.41-48. http://dx.doi.org/10.19027/jai.4.41-48

Yadav, G., Meena, D.K., Sahoo, A.K., Das, B.K. and Sen, R., 2020. Effective valorization of microalgal biomass for the production of nutritional fish-feed supplements. *Journal of Cleaner Production*, *243*, 118697. https://doi.org/10.1016/j.jclepro.2019.118697

Yudha, A.P., 2008. *Senyawa antibakteri dari mikroalga Dunaliella sp. pada umur panen yang berbeda*. Bachelor Thesis. Institut Pertanian Bogor, Bogor.

Zheng, M., Schideman, L.C., Tomasso, G., Chen, W.T., Zhou, Y., Nair, K., Qian, W., Zhang, Y. and Wang, K., 2017 Anaerobic digestion of wastewater generated from the hydrothermal liquefaction of *Spirulina*: Toxicity assessment and minimization. *Energy conversion and management*, *141*, pp.420-428. https://doi.org/10.1016/j.enconman.2016.10.034