TEST OF PROTEIN CONTENT IN PASTA OF *Nannochloropsis sp.* ISOLATED FROM LAMPUNG MANGROVE CENTER IN INTERMEDIATE SCALE CULTURE

Tugiyono[1], Agus Setiawan[2], Emy Rusyani[3], Ika Widyawati[1]

[1]Departmet of Biology, Faculty of Mathematics And Natural Sciences University of Lampung
[2]Department of Forest Management, Faculty of Agriculture University of Lampung
[3]Lampung Marine Culture Development Center
Email : tugiyono.1964@fmipa.unila.ac.id

ABSTRACT: *Nannochloropsis* sp. is a phytoplankton required in the ocean culture activities as living feed for fish larva. One of ocean with abundant *Nannochloropsis* sp. is the Lampung Mangrove Center. The objective of this research was to find out the protein content from *Nannochloropsis* sp. paste of isolate from Lampung Mangrove Center in the intermediate scale culture that was administered with combined fertilizer and different doses of NaOH as coagulant agent. This research used factorial completely random design with two treatments and each treatment was repeated three times. The first treatment was by seeing differences of fertilizer combination administrations between agricultural fertilizer (Urea 40 ppm, Za 20 ppm and TSP 5 ppm) and Conwy fertilizer 1 ml/L. The second treatment was paste making by administering different NaOH doses (100 ppm, 125 ppm, 150 ppm and 175 ppm). Data were analyzed by using analysis of variance (ANOVA), and whenever any significant difference result was obtained, then Least Significant Different test with $\alpha = 0.05$ was conducted. The research result showed that the highest protein content (15.73%) was produced by *Nannochloropsis* sp. paste in the Conwy fertilizer treatment combined with NaOH dose of 175 ppm.

Keywords: Nannochloropsis sp., protein, combination of fertilizer and NaOH dose

1. INTRODUCTION

Fish seeds or larva availability, both from quality and sustainability, should be balanced with the ocean fishery culture. Good quality feed availability, especially natural feed such as phytoplankton (micro algae) and zooplankton, today is difficult to provide, so that fish larva procurement can be hampered. The natural feed availability currently is still needed even though artificial feed has been widely produced for feeding fish larva. This is because natural feed has superiority than artificial feed that it can maintain the water quality and it has balanced nutrition [1].

Nannochloropsis sp. is one of phytoplankton kinds with high nutrition content, so that it can be used widely as feed for hatchery aquaculture industries such as fish larva, bivalvia juvenile, and rotifer [2]. The nutrition content obtained from proximate analysis on *Nannochloropsis* sp. administered with Conwy fertilizer showed 17.25% protein, 32.42 carbohydrate, and 4.13% fat [3].
Mangrove forest ecosystem is a place for varying micro algae that are able to grow and develop so that they are potential for being industrial biotarget [4] (Bahtiar, 2007). The analysis result of

gut contents from 13 types of fishes taken from Lampung Mangrove Center showed three types of algae with the most population; they were *Nannochloropsis* sp., *Tetraselmis* sp. and *Nitzchia* sp.[5]. The most occurring problem is the sustainable *Nannochloropsis* sp. availability, because it is difficult to culture in mass volume because of lack of sun rays in the rainy season and the environmental changes. Decreasing zooplankton (rotifer) population is because of reduced *Nannochloropsis* sp. density, and this affects the decreasing of fish larva population [6]. Therefore, an effort to overcome that reducing micro alga population must be done.

One of method had found in order to make a biomass of micro algae coagulates into solid matter (paste) that can be used as rotifer natural feed. NaOH that is administered to the culturing media shall improve the pH value in the water, so that microalgae shall undergo coagulation . The *Nannochloropsis* sp. cells shall stick and coagulate in the high pH condition [7,8].
The pure isolate stock making from three types of phytoplankton (*Nannochloropsis* sp., *Tetraselmis* sp., and *Nitzschia* sp.) obtained from Lampung Mangrove Center had been done in the previous research. According to [5], the *Nannochloropsis* sp. is one of three types of phytoplankton such as *Tetraselmis* sp. and *Nitzschia* sp. that has best growth acceleration and population density based on laboratory culture results. Therefore, a further research concerning the *Nannochloropsis* sp. paste making and to find out the nutrition content, especially protein, in the *Nannochloropsis* sp. paste isolated from Lampung Mangrove Center in intermediate scale should be carried out.

2. MATERIAL AND METHOD

This research has been conducted from August to November 2018 in phytoplankton laboratory, Natural Feed Division, Ocean Fishery Culture Office, in Hanura village of Teluk Pandan sub district in Pesawaran district.

Equipment to use in this research included aquarium, dropping pipette, plankton net, aeration equipment, refractometer, pH meter, microscope, thermometer, hemocytometer, hand counter, analytical balance, glass bottle, culture selves, sterilization equipment, mortar and pestle, filter, stir, aluminum foil, aquarium cover, and light meter.
Materials to use included *Nannochloropsis* sp. isolate from Lampung Mangrove Center, Conwy technical fertilizer, urea fertilizer, ZA, TSP, Vitamin B12, alcohol 70%, sterile ocean water, aquadest, aquabidest, NaOH, fresh water, chlorine 100 ppm and iodine.

This research used factorial completely randomized design in intermediate scale with 100 liter volume. There were two treatments and each treatment were repeated three times. The first treatment was the agricultural fertilizer combination of urea, ZA, TSP (40 ppm, 20 ppm and 5 ppm) and the second treatment was administration of 1 mL/L Conwy (CW) fertilizer.
The growth data were analyzed by using one way analysis of variance (ANOVA) and Least Significant Different with α=0.05 was conducted if any difference was found. Nutrition content data were analyzed descriptively.

Nannochloropsis sp. Culture

Nannochloropsis sp. culturing was started by the preparation stage that included ocean water sterilization and materials, fertilizer making, culturing seeds of *Nannochloropsis* sp. isolate from Lampung Mangrove Center by using fertilizer composition according to research treatments. After seeds were sufficient, culture was removed to a semi mass scale with initial inoculum density of 50 x 10^4 cell/mL and administered with fertilizer according to treatments. Parameters

to observe included population density, specific growth rate, generation time, and protein content.

The population density estimation was performed in each 24 hours during 7 days by using hemocytometer under a microscope. The calculating of population density as follows:

$$\sum \text{cell/mL} = N \times 10^4 \quad [9]$$

note
$\sum$ cell/ml : cell density
N : cell average numbers

After population density data were obtained, the specific growth rate can be estimated by using the following formula:

$$K = \frac{\text{Ln } W_t - \text{Ln } W_o}{T} \quad [10]$$

note
K : specific growth rate (cell/mL/day)
T : culturing time from W_o to W(day)
W_o : initial cell numbers (cell /mL)
W_t : cell numbers after time (cell/mL)

The generation time is estimated by using a formula as follow:

$$\frac{T}{3{,}3\ (\log Wt - \log Wo)} \quad [11]$$

Note **G =**
G : generation time (hour)
T : time from W_o to W_t (hour)
Wt : cell numbers after time t (cell/mL)
Wo : initial cell numbers (cell/mL)

Making Pasta of *Nannochloropsis* sp.

The *Nannochloropsis* sp. pasta making was started with preparation stage for materials and equipment. NaOH was weighed according to the doses to use; 100 ppm, 125 ppm, 150 ppm and 175 ppm. The weighing result was entered into small sized zipped plastic bags. For the *Nannochloropsis* sp. seeds, after 5 days of intermediate scale culturing, the *Nannochloropsis* sp. seeds were ready to make for paste by diluting them with NaOH that had been prepared by using sterile water in a small sized glass bottle and then it was stirred up until homogeneous. The solution was entered slowly into the *Nannochloropsis* sp. culturing media and then homogenized by stirring up and assisted with aeration. The *Nannochloropsis* sp. culturing media that had been homogenized with NaOH was left aside for 24 hours to sediment. The *Nannochloropsis* sp. culturing media was covered with tarpaulin plastic cover to prevent sunrays and outside weather. After sedimentation, the water in *Nannochloropsis* sp. culturing media was removed by using a hose so that only *Nannochloropsis* sp. sediment was left. The *Nannochloropsis* sp. sedimentation was entered into a 2000 mL glass jar and then moved into filters with satin cloth functioning to filter and each filter was labelled and they were left aside for 24 hours to obtain *Nannochloropsis* sp.

paste. After 24 hours, the paste was entered to plastic containers and labelled according to determined codes. The paste in the plastic container was weighed to find out paste weight for each different dose.
Nutrition observation with proximate analysis was done in water quality laboratory in the Ocean Fishery Culture Office in Lampung when the culture was in exponential phase. The proximate analysis was performed to see the protein content and protein analysis was performed by using Kjedahl semi-micro method.

3. RESULTS AND DISCUSSION

The results showed that the highest population density of each treatment occurred in the seventh day (Figure 1). The average of highest population occurred in the NaOH dose that was combined with Conwy technical fertilizer with population density of 3918.33 x 10^4 cell/mL, and then followed by 100 ppm of P with population density of 3826.66 x 10^4 cell/mL and then followed by 150 ppm of P with population density of 3615 x 10^4 sel/mL, treatment with 175 ppm of C with population density of 3373.33 x 10^4 cell/mL, administration of 125 ppm of P with population density of 3326.33 x 10^4 cell/mL, administration of 150 ppm of C with population density of 3240 x 10^4 cell/mL, administration of 175 ppm of P with population density of 3130 x 10^4 cell/mL, and the lowest population density was obtained in the treatment of 125 ppm of C with population density of 2683.33 x 10^4 cell/mL.

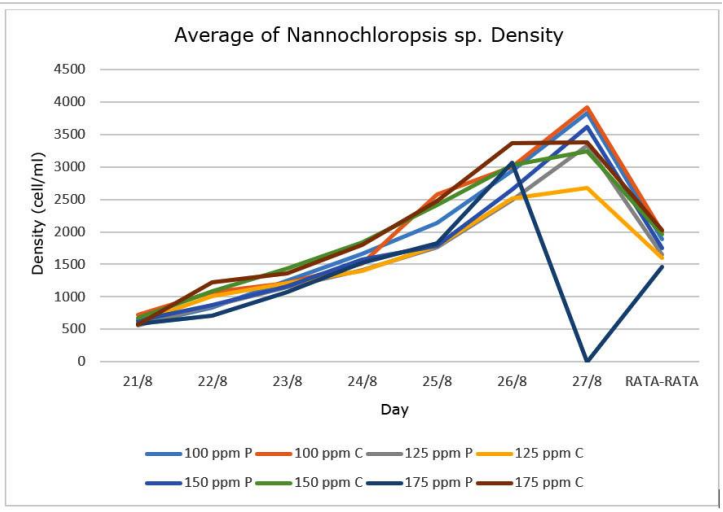

Figure 1. Graphic of average of population density of Nannochloropsis sp. in each treatment

Note:
100 ppm P : dose administration of NaOH 100 ppm with agricultural fertilizer combination (Urea, ZA and TSP)
100 ppm C : dose administration of NaOH 100 ppm with Conwy technical fertilizer.
125 ppm P : dose administration of NaOH 125 ppm with agricultural fertilizer combination (Urea, ZA and TSP)
125 ppm C : dose administration of NaOH 125 ppm with Conwy technical fertilizer
150 ppm P : dose administration of NaOH 150 ppm agricultural fertilizer combination (Urea, ZA and TSP)
150 ppm C : dose administration of NaOH 150 ppm with Conwy technical fertilizer
175 ppm P : dose administration of NaOH 175 ppm agricultural fertilizer combination (Urea, ZA and TSP)

175 ppm C : dose administration of NaOH 175 ppm with Conwy technical fertilizer

Graphic in Figure 1 shows adaptation phase in the administration of 175 ppm of C that occurs so short compared to other treatments, and this is because the seeds are obtained in exponential phase. In the second and seventh day the populations density keeps on increasing because of available nutrition is still sufficient for the *Nannochloropsis* sp. metabolism, so that *Nannochloropsis* sp. can still split the cell up to the day seven. The graphic shows that the administration of 175 ppm of both agricultural fertilizer combination and Conwy technical fertilizer have stationary phase in the day six, while other treatments do not undergo stationary phase. This is because stationary phase need time less than 24 hours so that it is not observed because any estimation is always done in 24 hours.

Table 1. Average of *Nannochloropsis* sp. Maximum Population Density in Each Treatment

Treatment	**Maximum Population Density (x 10^4 cell/mL) *Nannochloropsis* sp. (Mean)***
100 ppm P	3826.66
100 ppm C	3918.33
125 ppm P	3326.66
125 ppm C	2683.33
150 ppm P	3615
150 ppm C	3240
175 ppm P	3130
175 ppm C	3373.33

Growth Rate

The growth rate for 100 ppm of P administration until 175 ppm of C administration significantly differ between treatments (Table 2). The highest growth rate occurs in 100 ppm of P administration with 0.2705 cell/mL/day and followed by 125 ppm of P administration, and then 175 ppm of C, 150 ppm of P, 175 ppm of P, 100 ppm of C, 150 ppm of C and the last 125 ppm of C.

Table 2. Average of Specific Growth rate of *Nannochloropsis* sp.in Each Treatment

Treatment	**Specific Growth Rate (cell/mL/day) *Nannochloropsis* sp. (Mean)**
100 ppm P	0.2705
100 ppm C	0.2339
125 ppm P	0.2547
125 ppm C	0.2106
150 ppm P	0.2471
150 ppm C	0.2232
175 ppm P	0.2357
175 ppm C	0.2531

Generation Time

The generation time for treatment 100 ppm of P until treatment 175 ppm of C differ significantly between treatments (Table 3). The fastest generation time occurs in treatment 150 ppm of C, followed by treatment 100 ppm of C, 175 ppm of P, 150 ppm of P, 125 ppm of P, 175 ppm of C, and the last 100 ppm of P.

Table 3. Average of Generation Time of *Nannochloropsis* sp. in each treatment

Treatment	Generation Time (hour) *Nannochloropsis* sp.(Mean)
100 ppm P	25.97
100 ppm C	30.781
125 ppm P	27.874
125 ppm C	27.719
150 ppm P	28.250
150 ppm C	31.337
175 ppm P	30.161
175 ppm C	27.870

The generation time of treatment 100 ppm of P is 25.97 hour, it means that once cell splitting of *Nannochloropsis* sp. into two cells requires 25.97 jam. Based on the research result, the highest population density has the highest growth rate and the fastest generation time.

Paste Weight

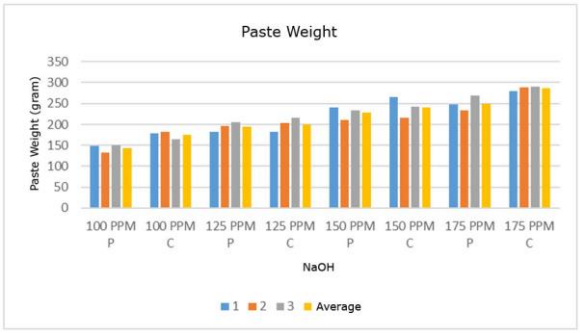

Figure 2. Weight of *Nannochloropsis* sp. Paste in Each Treatment

Figure 2 shows that the higher the NaOH dose, then the higher the paste weight shall be. The highest paste weight can be found in treatment with highest dose of NaOH; 175 ppm of C. the lowest paste weight can be found in the lowest NaOH dose; 100 ppm of P.

Protein Content

Treatment with 175 ppm of C has average highest percentage of protein content of 15.73%, followed by treatment with 150 ppm of C with 14.87% protein content, then 100 ppm of P with 13.64% protein content, 125 ppm of C with 12.81% protein content, 175 ppm of P with 12.75% protein content, 125 ppm of P with 12.69% protein content, 150 ppm of P with 12.52% protein content and the last 100 ppm of P with 11.44% protein content.

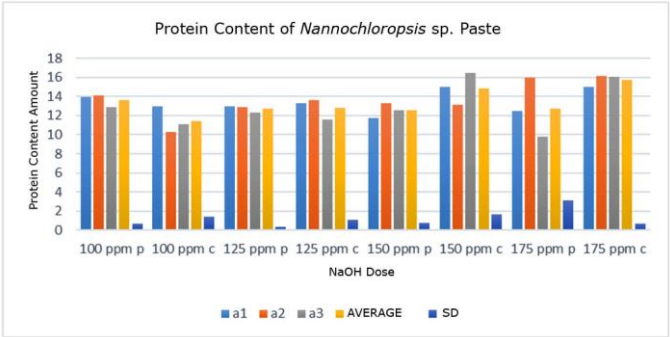

Figure 3. Graphic of *Nannochloropsis* sp. Proetin Content in Each Treatment.

According to Stickney [12] (2005), protein is the main component and very important for both animal and human cells. This is because protein has a role as a main substance in boy formation. Protein also becomes an important enzyme responsible for catalyzing thousands of biochemical reactions. According to [13] Poedjiadi (1994), protein is a polypeptide having different characteristics with varying weights. Some of proteins are soluble in water and some others are insoluble in water. The basic structures of protein are primary structure, secondary structure, tertiary structure and quartet structure.

4. CONCLUSION

Based on the research results, the conclusions are as follows:

1. The higher the NaOH dose, then the higher the *Nannochloropsis* sp. paste weight shall be.
2. The highest protein content percentage of 15.73% occurs in NaOH dose of 175 ppm combined with Conwy technical fertilizer.

5. ACKNOWLEDGEMENTS

We would like to express our gratitude to the the Directorate of Research and Community Services, Directorate General of Higher Education (DIKTI), Ministry of Research, Technology and Higher Education of the Republic of Indonesia (Kemenristekdikti) for the research grant through the the intitutional national strategic grant scheme with the contract number:393/UN26.21/PN/2018. We also would like to thank the Head of Lampung Marine Culture Development Center for permitting this research.

6. REFERENCES

[1] Widjaja, F, Pendayagunaan *Rotifera* yang Diberi Pakan Alami Jenis Mikroalgae. Jurnal Ilmu – Ilmu Perairan dan Perikanan. Institute Pertanian Bogor, 2004.
[2] Tawfiq A. S., Al-musallam L., Al- shimmari J and Dias P, Optimum production conditions for different high-quality marine algae, Hydrobiologia 403, 97-107, 1999.
[3] Rusyani, E., Laporan Tahunan. Balai Besar Pengembangan Budidaya Laut. Lampung, 2012.
[4] Bahtiar, E., Penelusuran Sumber Daya Hayati Laut (Alga) sebagai Biotarget Industri. Fakultas Perikanan dan Ilmu Kelautan Universitas Padjadjaran. Jatinangor,2007.

[5] Tugiyono, J. Master, and Suharso, "Isolation and identification of phytoplankton from aquatic ecosystemsof Lampung Mangroves Center (LMC) as biological feed," Asian J. Agri & Biol. Vol 5, no.4, pp.188-194, 2017.
[6] Muliono, A., Pengaruh Suhu dan Lama Penyimpanan Terhadap Kondisi Sel *Nannochloropsis* sp. Skripsi. Fakultas Perikanan dan Ilmu Kelautan, IPB, 2004.
[7] Kokarkin, C. Dan E. Kusnendar. Marine Microalgae Engineering With a Special Emphasis on *Chlorella* sp. and its Potensial Use in the Future (Proceadings of the International Symposium on Marine Biotechnology, Ancol, Jakarta 29-31 Mei 2000), Jakarta.2000.
[8] Sukarni, Sudjito, Hamidi N,Yanuhar Uun, Wardana, I.N.G, 2014. Potential and properties of marine microalgae Nannochloropsis oculata as biomas fuel feedstock. Int.J Energy Environ Eng 5:279-290. DOI 10.1007/s40095-014-0138-9,2014.
[9] Mudjiman, "*Makanan Ikan"* Edisi Revisi, PT. Penebar Swadaya, Jakarta. 2007.
[10] Fogg, G.E., *Algal Cultures and Phytoplankton Ecology*. The University of Wisconsin press. London, 1987.
[11] Kurniastuty dan Julinasari, Pertumbuhan alga *Dunaleilla* sp. pada media kultur yang berbeda dalam skala masal (semi outdoor). *Buletin Budidaya Laut Lampung*. No 9.1995
[12]Stickney,R.R., Aquaculture: An Introductory Text, CABI Publishing, USA, 256p, 2005.
[13] Poedjiadi Anna, Dasar-dasar Biokimia. UI Press. Jakarta, 1994.