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The endurance test of *Nannochloropsis* sp. paste isolated from Lampung Mangrove Centre (LMC)

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The endurance test of *Nannochloropsis* sp. paste isolated from Lampung Mangrove Centre (LMC)

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Abstract. *Nannochloropsis* sp. has been used as natural feed because it has high nutrition that is good for fish larva growth and development. However, the *Nannochloropsis* sp. availability continually is in insufficient amount and this often becomes a problem in culturing because this is difficult to culture in mass volume. The objective of this research was to make *Nannochloropsis* sp. paste and to test the paste quality based on the life endurance of *Nannochloropsis* sp. cells from isolate of Lampung Mangrove Centre in intermediate scale culture by using different fertilizer combination and NaOH doses. This research used factorial completely randomized design with two treatments and three repetitions. The first treatment was administration of agricultural fertilizer combination of (P) urea 40 ppm, ZA 20 ppm, and TSP 5 ppm, and Conway (C) technical fertilizer as control. The second treatment is the administration of NaOH doses of: 100 ppm, 125 ppm, 150 ppm, and 175 ppm. Data were analyzed by using One Way Analysis of Variance (ANOVA), and Least Significant Difference test with $\alpha = 0.05$ was performed whenever any significant difference was found. The research result showed that *Nannochloropsis* sp. paste from isolate of Lampung Mangrove Centre had highest life endurance level with Conwy technical fertilizer and dose of NaOH 100 ppm with population density of 18066666.67 x 10^4 cell/mL.

1. Introduction

Based on Decree of East Lampung regent No.660/305/04/SK/2005/1546/J. 26/KL/2005 in 10 May 2005, Lampung Mangrove Centre is one of mangrove ecosystems in Lampung province, and it is located in Margasari village of Labuhan Maringgai sub district in East Lampung district with 700 Ha area width [1] (monography of Margasari village, 2005). Lampung Mangrove Centre as an ecosystem becomes one of feed sources for living creatures such as fish and shrimp larvae that contain of phytoplankton and zooplankton.

Currently, phytoplankton as a natural feed source is very needed in culturing, especially in fish hatchery activities. However, feed necessity often becomes a problem because this feed availability is often less than sufficient. There are two plankton kinds for natural feed; phytoplankton and zooplankton. Phytoplankton is the organism producing micro algae [2,3]. There are three kinds of

phytoplankton most often found in Lampung Mangrove Centre water for fish feed; *Nannochloropsis* sp. *Tetraselmis* sp. and *Nitzchia* sp. [4].

Nannochloropsis sp. has been used for natural feed because it has high nutrition that is good for fish and shrimp larva growth and development, and in addition, this micro alga is easy to grow in varying environment conditions [5]. The *Nannochloropsis* sp. availability in continual manner often becomes a problem in culturing, because of environmental change factors and less sunrays in rainy season, so that *Nannochloropsis* sp. is difficult to be mass cultured [6].

The effort to provide phytoplankton population is performed by producing *Nannochloropsis* sp. paste. This paste making is a practical way to provide natural feed stock. The paste making is performed by precipitating *Nannochloropsis* sp. by adding NaOH in culturing media, so that pH value in water can improve [7,8]. Therefore, a research concerning paste making by using fertilizer and NaOH with different doses with an objective to find out the life endurance of *Nannochloropsis* sp. isolate from Lampung Mangrove Centre was performed.

2. Research method

This research was conducted from August to November 2018 in zooplankton laboratory of Living Feed Division, in Ocean Fishery Culture Office in Lampung, located in Jalan Yos Sudarso, in Hanura village of Teluk Pandan sub district, Pesawaran district, Lampung province.

Equipment to use in this research included aquarium, aquarium jar, table, droplet pipette, hemocytometer, satin cloth, microscope, glass cover, Erlenmeyer flask, measuring cup, scale, spatula, plastic spoon, plastic disc, beaker glass, plastic hose, hand counter, plastic funnel, filtering cloth, magnetic stirrer, plastic container, thermometer, DO meter, spectrophotometer, pH meter, and refractometer. Materials to use in this research included *Nannochloropsis* sp. seeds from Lampung Mangrove Centre, agricultural fertilizer (urea, ZA, TSP), Conwy technical fertilizer, Vitamin B12, NaOH, sterile sea water, vidone, citric acid, aquabidest, alcohol 70%, fresh water, disk washing detergent and chlorine solution.

This research used experimental method with factorial completely randomized design with two treatments and each treatment was repeated three times. The first treatment was 12 aquariums administered with combined agricultural fertilizer (P) or Urea 40 ppm, ZA 20 ppm, and TSP 5 ppm; and other 12 aquariums were administered with Conwy technical fertilizer (C) 1 ppm as control. The second treatment was administration of NaOH doses; 100 ppm, 125 ppm, 150 ppm, and 175 ppm.

Data obtained in this research were presented in tables and graphics. Data of cell density and cell daily growth rate of *Nannochloropsis* sp. isolate from Lampung Mangrove Centre were presented in tables and graphics and were analyzed by using descriptive analysis. Data analysis was done by using One Way Analysis of Variance (ANOVA) and Least Significant Difference test with 95% level (α = 0.05) was conducted whenever any significant difference was found.

2.1. Preliminary research

2.1.1. Cluturing Nannochloropsis sp. Isolate from Lampung Mangrove Centre. The culturing of *Nannochloropsis* sp. isolated from Lampung Mangrove Centre (LMC) was done in intermediate scale (semi-mass). Stages in culturing *Nannochloropsis* sp. were as follows: the *Nannochloropsis* sp. seeds from LMC was cultured by using Erlenmeyer flask that would be used in the intermediate scale culturing. The *Nannochloropsis* sp. seeds were removed into intermediate scale by using 1000 liter volume aquarium. The seeds of *Nannochloropsis* sp. from LMC was cultured in the aquarium and re-cultured again up to 24 aquariums. The seeds of *Nannochloropsis* sp. from LMC were removed from aquarium into one container of 1 m^3 and harvested. The initial density of *Nannochloropsis* sp. seeds were counted by using hemocytometer. The initial seed density for spreading and volume of sea water in each aquarium were calculated by using the following formula [9]:

$$\mathbf{V1 \times N1 = V2 \times N2} \quad (1)$$

Note:
V1 = seed volume for initial spreading (mL)
V2 = desired volume of *Nannochloropsis* sp. culture media (mL)
N1 = seed density / stock of *Nannochloropsis* sp. (cell/mL)
N2 = desired seed density of *Nannochloropsis* sp. from LMC (cell/mL)

24 aquariums were filed with sterile sea water and Conwy technical fertilizer was administered into 12 aquariums and agricultural fertilizer (urea, ZA, and TSP) was administered into other 12 aquariums. Testing for measuring salinity, temperature, DO, and light intensity were performed. Water quality test was performed in the beginning and at the end of treatment. Water quality testing was performed in water quality laboratory in Ocean Fishery Culture Office in Lampung. The measurements were performed to measure: nitrite, nitrate, phosphate, ammoniac, and pH. Cell density of *Nannochloropsis* sp. from LMC in 24 aquariums were counted

2.1.2. Nannochloropsis sp. Paste Making. Stages in *Nannochloropsis* sp. paste making were as follows. NaOH doses were weighed; 100 ppm, 125 ppm, 150 ppm and 175 ppm. NaOH was dissolved in the bottle and then poured into aquariums containing *Nannochloropsis* sp. from LMC. The aquarium content was stirred up slowly until homogenous and it was helped with aeration for precipitating process. The aquariums were covered with tarpaulin to prevent sunrays and then left aside for 24 hours. After 24 hours, the *Nannochloropsis* sp. was harvested by filtering it. Precipitated deposit of *Nannochloropsis* sp. was removed from aquarium into filtering container for 24 hours to obtain *Nannochloropsis* sp. paste that was free from water. The *Nannochloropsis* sp. paste was then entered into plastic bags and weighed for each bag, and then they were labelled according to NaOH doses.

2.2. Main Research
Stages in the main research were as follows. Sterile sea water, 24 aquariums and *Nannochloropsis* sp. paste seeds from LMC in the preliminary research were prepared. 5% citric acid was weighed and dissolved into the sterile sea water. *Nannochloropsis* sp. paste seed was entered into a container and filtered by using filtering cloth and then dissolved by using sterile sea water. Each sample of *Nannochloropsis* sp. seed was administered with 5% citric acid concentration. 24 culture containers were arranged and randomized by using factorial completely randomized design with two treatments and each treatment was repeated three times. The containers were filled with sea water and aerated, given with agricultural fertilizer, Conwy fertilizer, and Vitamin B12 2 mL. measurements for salinity, temperature, DO, and light intensity were performed. Water quality test was performed in the beginning and at the end of treatment. Containers were covered with covered with aluminum foil to prevent evaporation and then cell density was counted every 24 hours.

3. Result and Discussion

3.1. Nannochloropsis sp. Paste weight
Data of *Nannochloropsis* sp. paste weight in each treatment can be seen in Figure 1.

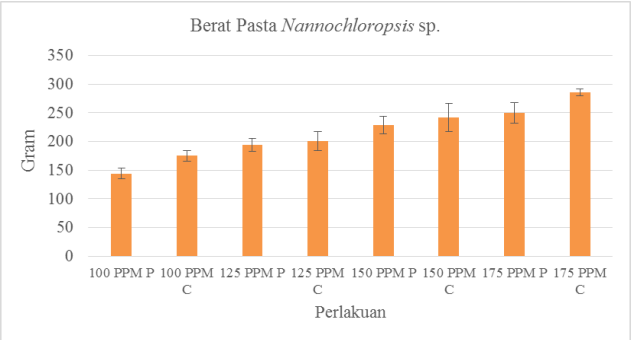

Figure 1. Graphic of *Nannochloropsis* sp. paste in each treatment

Figure 1 above shows that the highest average of *Nannochloropsis* sp. paste weight in this research is produced by treatment of 175 ppm of C (286 grams).

Note:
100 PPM P = treatment of agricultural fertilizer and NaOH 100 ppm dose
100 PPM C = treatment of Conwy technical fertilizer and NaOH 100 ppm dose
125 PPM P = treatment of agricultural fertilizer and NaOH 125 ppm dose
125 PPM C = treatment of Conwy technical fertilizer and NaOH 125 ppm dose
150 PPM P = treatment of agricultural fertilizer and NaOH 150 ppm dose
150 PPM C = treatment of Conwy technical fertilizer and NaOH 150 ppm dose
175 PPM P = treatment of agricultural fertilizer and NaOH 175 ppm dose
175 PPM C = treatment of Conwy technical fertilizer and NaOH 175 ppm dose

3.2. Nannochloropsis sp. population density

In the endurance life test of paste of *Nannochloropsis* sp. isolate from Lampung Mangrove Centre (LMC) in the intermediate scale, culturing was performed for 5 research days and the population density was counted from 4 September to 8 September 2018 in every 24 hours. The research result shows that the highest *Nannochloropsis* sp. population density for each treatment is obtained in third day of research (Figure 2). The highest average population density is in treatment 10 ppm of C with population density of 18066666.67 x 10^4 cell/mL, then followed by treatment 125 ppm of P with population density of 17950000.00 x 10^4 cell/mL, treatment 125 ppm of C with population density of 16883333.33 x 10^4 cell/mL, treatment 150 ppm of P with population density of 14166666.67 x 10^4 cell/mL, treatment 100 ppm of P with population density of 13783333.33 x 10^4 cell/mL, treatment 150 ppm of C with population density of 12783333.33 x 10^4 cell/mL and treatment 175 ppm of P with population density of 9516666.67 x 10^4 cell/mL, and the lowest is in treatment 175 ppm of C with population density of 8233333.33 x 10^4 cell/mL.

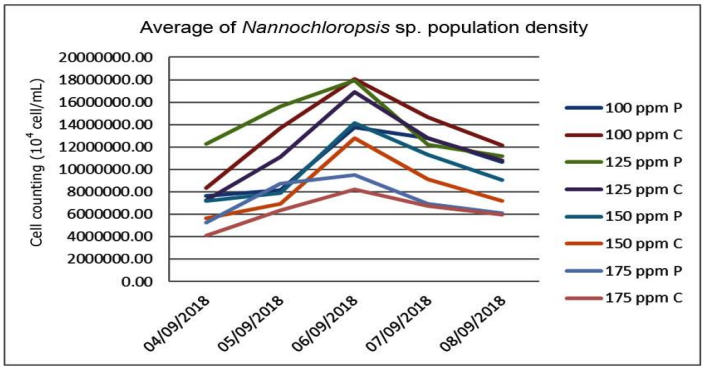

Figure 2. Graphic of average of *Nannochloropsis* sp. density in each treatment

note:
100 ppm P = treatment of agricultural fertilizer and NaOH 100 ppm dose
100 ppm C = treatment of Conwy technical fertilizer and NaOH 100 ppm dose
125 ppm P = treatment of agricultural fertilizer and NaOH 125 ppm dose
125ppm C = treatment of Conwy technical fertilizer and NaOH 125 ppm dose
150 ppm P = treatment of agricultural fertilizer and NaOH 150 ppm dose
150 ppm C = treatment of Conwy technical fertilizer and NaOH 150 ppm dose
175 ppm P = treatment of agricultural fertilizer and NaOH 175 ppm dose
175 ppm C = treatment of Conwy technical fertilizer and dosis NaOH 175 ppm dose

Graphic in Figure 2 shows that the first and second day of treatment show slow adaptation phase toward administration of combination of fertilizer and NaOH. It is suspected that the seeds underwent longer adaptation phase before coming into exponential phase. In addition, the adaptation phase went longer because extreme temperature in the first and second day of treatment that caused death some of cells while some others proceed to make adaptation by stopping cell splitting. [10] explain that too high sunrays intensity can inhibit phytoplankton growth rate. In addition, [11] also explains that *Nannochloropsis* sp. micro alga culture in optimal temperature shall produce good population development.

In the third day, the population density kept on increasing or entered the exponential phase where in this phase the *Nannochloropsis* sp. performed cell splitting and reached peak of population density or exponential phase. This can occur because nutrition and water quality of culture media are still supportable for the growth and cell splitting of *Nannochloropsis* sp. [12].

In the fourth dan fifth day of treatment, *Nannochloropsis* sp. growth underwent decrease, and it may occur because worsening water quality and nutrition runs out so that *Nannochloropsis* sp. cannot do more metabolism to support its growth. Cell population density decreases fastly because the death rate of phytoplankton is higher than its growth rate [13]. This was affirmed with color change in culture media into clear and *Nannochloropsis* sp. precipitation in the bottom of aquarium had sandy texture and was unable to dissolve again when being stirred up [14]. The graphic in Figure 2 does not show stationary phase, and it is suspected that the stationary phase occurred in less than 24 hours while observation of population density was performed in each 24 hours. Therefore, the stationary phase was unobservable and death phase was directly observed after exponential phase.

The graphic in Figure 2 above shows that the average cell life endurance of *Nannochloropsis* sp. isolate from Lampung mangrove Centre (LMC) that is able to produce highest average of 18066666.67 x 10^4 cell/mL population density is treatment 100 ppm of C, while the lowest is treatment 175 ppm of C with 8233333.33 x 10^4 cell/mL population density.

3.3. Growth rate

Data of *Nannochloropsis* sp. specific growth rate calculation result can be seen in Table 1. Table 1 shows that the highest *Nannochloropsis* sp. growth rate occurs in treatment 100 ppm of C with 0.15 cell/mL/day, and then followed by treatment 150 ppm of C with 0.14 cell/mL/day, treatment 175 ppm of C with 0.14 cell/mL/day, treatment 125 ppm of C with 0.13, treatment 175 ppm of P with 0.12 cell/mL/day, treatment 150 ppm of P with 0.11 cell/mL/day, treatment 100 ppm of P with 0.08 cell/mL/day, and the last treatment 125 ppm of P with 0.06 cell/mL/day.

Table 1. Average of *Nannochloropsis* sp. specific growth rate in each treatment

Treatments	Specific growth rate (cell/mL/day) *Nannochloropsis* sp. (Mean ± Standar Eror)
100 ppm P	0.08 ± 0.02[a]
100 ppm C	0.15 ± 0.03[a]
125 ppm P	0.06 ± 0.02[a]
125 ppm C	0.13 ± 0.02[a]
150 ppm P	0.11 ± 0.02[a]
150 ppm C	0.14 ± 0.04[a]
175 ppm P	0.12 ± 0.02[a]
175 ppm C	0.14 ± 0.05[a]

Note:
The same superscript letter in the same column states no significant difference at α = 0.05

Note:
100 ppm P = treatment with agricultural fertilizer and NaOH 100 ppm dose
100 ppm C = treatment with Conwy technical fertilizer and NaOH 100 ppm dose
125 ppm P = treatment with agricultural fertilizer and NaOH 125 ppm dose
125ppm C = treatment with Conwy technical fertilizer and NaOH 125 ppm dose
150 ppm P = treatment with agricultural fertilizer and NaOH 150 ppm dose
150 ppm C = treatment with Conwy technical fertilizer and NaOH 150 ppm dose
175 ppm P = treatment with agricultural fertilizer and NaOH 175 ppm dose
175 ppm C = treatment with Conwy technical fertilizer and NaOH 175 ppm dose

3.4. Generation time

Generation time is the time required by micro alga to split cell, from one cell into two cells [15]. The calculation for generation time of each treatment can be seen in Table 2.

Table 2. Average generation time of *Nannochloropsiss* sp. in each treatment

Treatments	Generation time (hour) *Nannochloropsis* sp. (Mean ± Standar Eror)
100 ppm P	11.75 ± 2.64[a]
100 ppm C	16.39 ± 5.45[a]
125 ppm P	7.68 ± 2.41[a]
125 ppm C	6.37 ± 1.08[a]
150 ppm P	10.38 ± 2.75[a]
150 ppm C	5.47 ± 0.83[a]
175 ppm P	16.75 ± 12.99[a]
175 ppm C	17.31 ± 11.71[a]

Note:
The same superscript letter in the same column states no significant difference at α = 0.05
100 ppm P = treatment with agricultural fertilizer and NaOH 100 ppm dose
100 ppm C = treatment with Conwy technical fertilizer and NaOH 100 ppm dose
125 ppm P = treatment with agricultural fertilizer and NaOH 125 ppm dose
125ppm C = treatment with Conwy technical fertilizer and NaOH 125 ppm dose
150 ppm P = treatment with agricultural fertilizer and NaOH 150 ppm dose
150 ppm C = treatment with Conwy technical fertilizer and NaOH 150 ppm dose
175 ppm P = treatment with agricultural fertilizer and NaOH 175 ppm dose
175 ppm C = treatment with Conwy technical fertilizer and NaOH 175 ppm dose

The fastest generation time is obtained by treatment 175 ppm of C (treatment with Conwy technical fertilizer and NaOH 175 ppm dose) that reaches 17.32. This indicates that the time required by one cell of *Nannochloropsis* sp. to split into two is 17.32 hours. The second fastest generation time is 16.75 hours by treatment 150 ppm of C, followed by 16.39 in treatment 125 ppm of P, 11.76 hours by treatment 100 ppm of P, 10.38 hours by treatment 100 ppm of C, 7.69 hours by 150 ppm of P, 6.37 hours by treatment 175 ppm of P, and the last 5.48 hours by treatment 150 ppm of P.

3.5. Water quality

Water quality data were measured in the beginning and in the end of *Nannochloropsis s*p. culturing. Measured parameters included temperature, DO, pH, salinity, light intensity, nitrite, nitrate, phosphate and ammoniac. Water quality data during the research are presented in attachment 5.

Water quality data indicated that parameters influencing the *Nannochloropsis s*p. growth were temperature, salinity, light intensity, DO, pH, and ammoniac [16,17].

Temperature is an important factor influencing micro alga growth. In this research, temperature change in media culture was influenced by light intensity. The optimal range of temperature for *Nannochloropsis s*p. micro alga is 25 – 35 °C [18]. The temperature measurement during the research showed 29 ^{0}C, so that this temperature level became one of *Nannochloropsis s*p. growth inhibitors.

According to [18], the optimal salinity range for *Nannochloropsis s*p. growth is 25-35%. The salinity during research was 28%. It means that the salinity during the research is still proper for optimal growth of *Nannochloropsis s*p., and shows that salinity does not become inhibitor factor.

According to [19], light is the main energy source for micro alga to do photosynthesis. In a water ecosystem, micro alga can do assimilation process of organic materials provided that light necessity is fulfilled properly. Optimal light intensity for *Nannochloropsis s*p. micro alga growth is 2000-8000 lux. Too high light intensity can inhibit photosynthesis. Light intensity during this research was 9000 lux, and this was inconsistent with [19].

During the research, the pH value changes in culture media range 8.27-8.35, and these were still proper for the *Nannochloropsis s*p. growth, because it was in accordance with [16] suggesting that *Nannochloropsis s*p. could live in pH range of 8.0-9.5. In the condition of range pH 6-8 *Nannochloropsis s*p. and other microalgae generally can be applied as adsorbent for heavy metal ions [20,21,22,23,24,25,26].

4. Conclusion

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

1. The treatment of Conwy technical fertilizer and NaOH 175 ppm dose produces the most paste.
2. The paste of *Nannochloropsis s*p. isolate from Lampung Mangrove Centre shows highest life endurance in treatment of Conwy technical fertilizer and NaOH 100 ppm dose with population density of 18066666.67 x 10^4 cell/mL.

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