Modification of live feed from intermediate cultivation of isolate *Nannochloropsis* sp. of the waters of Lampung Mangrove Center

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The availability of live feed is an important role in aquaculture. *Nannochloropsis* sp. is a micro algae that can be used as the live feed for larvae cultivation of shrimp, fish, and shellfish. In this study, a *Nannochloropsis* sp culture was isolated from local waters by using agricultural fertilizer as a substitute for pro analyze fertilizer (Conway). The aims of this research were to investigate the cell density (cell/L), the growth rate of cell, and doubling time of isolates *Nannochloropsis* sp from the waters of Lampung Mangrove Center, which were cultured intermediately with the volume of 100 L. This research was using completely random design, with combination treatment of agricultural fertilizer (A: Urea 40, ZA 20, TSP 5 ppm, B: Urea 40, ZA 20, TSP 10 ppm, C: Urea 40, ZA 20, TSP 15 ppm, D: Urea 40, ZA 20, TSP 20 ppm, and E: Conwy and vitamin B12 dose 1 ppm (control)), with 4 replications. The results showed that the combination of the agricultural fertilizer resulted in A treatment, yielded the highest cell density rate and the fastest growth rate ($p < 0.050$) compared with the treatment of the others. In addition the treatment of A showed the fastest doubling time significantly ($p < 0,050$) to B, D, and E treatments, but it is not significant difference with the treatment of C ($p = 0,065$). The combination of the agricultural fertilizer treatment A is the best for growth rate of *Nannochloropsis* sp in intermediate culture.

Keywords: *Nannochloropsis* sp., intermediate scale culture, agricultural fertilizer, Lampung Mangrove Center.

Introduction

Natural feed availability (phytoplankton and zooplankton) in normal condition in natural water ecosystem sufficiently available and even abundant, and it can be used by each trophic level efficiently, especially fishes which belong to the top trophic level. The problem of natural feed necessity usually arises when organisms live in cultivation environment. Microalga has an important role in aquaculture, because microalga is the early level of food chain (Lubián, 1982).

Microalga has an ability to grow rapidly and to synthesize macro molecules (for example: carbohydrate, protein, and lipid) and chemical substances (for example: carotenoids, phycobilins, polyunsaturated fatty acids, and water-soluble polysaccharides). Mass culture of microalga in commercial scale was started about fifty years ago and microalga biomass has been used for functional food and nutraceuticals (astaxanthin, betacarotene, poly unsaturated) and additional cattle feed, especially aquaculture (as protein and mineral sources) (Hu, 2014; Sivakumar and Rajendran, 2013).

Microalga is expected to have an important role in aquaculture because it can be used directly for living feed for molluscan and crustacean larva or indirectly as feed for zooplankton species such as *Brachiomus plicatilis* and nauplii artemia (Barclay and Zeller, 1996). Microalga (phytoplankton) should meet some criteria to able to use in aquaculture including easy to culture, not toxic, nutrition values in accordance with cellular sizes, and cellular walls that are easy to digest to get available nutrition (Hemaiswarya et al., 2010).

Some microalge strains are usually used in aquaculture, based on the nutrition demands. The most common species are Nannochloropsis, Isochrysis, Scenedesmus, Dunaliella, *Spirulina phaeodactylum*, Pavlova, Tetraselmis, Skeletonema, Chlorella dan Thalassiosira (Pulz and Gross, 2004). *Nannochloropsis* sp is more familiar to known as sea chlorella and it is cultivated in semi-mass or mass scales for zooplankton feed such as *Barchionus plicatilis* or Rotifer, shell larvae or sea cucumber (Tawfiq, et al, 1999; Lubian et al., 2000).

In the water cultivation, especially hatcheries of fish, shell, and sea cucumber, microalga culture technique (phytoplankton and zooplankton) is an aspect must be mastered to support sustainable natural food stock availability. Plankton culture technique in common consists of three stages; the laboratory stage, semi-mass or mass scale, or open pond (Borowitzka, 2005).

Nannochloropsis sp is a sea microalga having high nutrition value, and it is used widely as aquaculture hatchery industry food such as larvae and juvenile of bivalvia, rotifer and fish larvae (Lubián, 1982; Tawfiq, et al, 1999). Microalga powder is used as substitution for aqua feed, for example, *Nannochloropsis* sp is used to enrich aqua feed for *Octopus vulgaris* paralarvae (Fuentes et al., 2011). The same thing is demonstrated by the high survival rate of *Sparus aurata* larvae being feed with microalga biomass compared to ordinary feed (Robin and Vincent, 2003).

Nannochloropsis sp has a characteristic to be easy to cultivate in semi-mass and mass cultivations and it is not toxic and it has antibiotic contents (Fulks and Main, 1991). In the semi mass or mass culture scales, the pro-analis fertilizer (Conway) causes very high cost, so that various agricultural fertilizers are used as sources of nitrogen, phosphate, and micronutrients as alternatives to reduce cost in producing microalga (Lam and Lee, 2012). Urban wastes are used for *Oedogonium* sp microalga culture and it is very potential to produce biocrude (Neveux, 2016).

The objective of this research was to find out the combination use of agricultural fertilizers for *Nannochloropsis* sp in semi-mass scale as an effort to seek proper dosage combination of agricultural fertilizers for *Nannochloropsis* sp growth.

Materials and Research Method

This research used Completely Randomized Design consisting of 5 treatments of agricultural fertilizers and Conway fertilizer was used as the control. Each treatment was repeated 4 times. The fertilizer concentrations are presented in Table 1.

Table 1. Fertilizer concentrations used in the research

Treatment	Agricultural Fertilizer Concentration (ppm)			Conway fertilizer as control (mL)
	Nitrogen (Urea) (Daefi,et al. 2017)	Ammonium Sulphate (Za) (Rusyani et al., 2007)	Triple Super Phospate (TSP)	
A	40	20	5	-
B	40	20	10	-
C	40	20	15	-
D	40	20	20	-
E (Control)	-	-	-	80

The inoculum of *Nannochloropsis* sp was collected from isolation result from Lampung Mangrove Center water area (Daefi et al., 2017), and then reproduced in the laboratory scale to get *Nannochloropsis* sp inoculum stock. In the intermediate scale culture, the initial *Nannochloropsis* sp inoculum density was 500 x 10^4 cell/mL with volume culture of 80 L placed in a 100 L aquarium (Rusyani et al, 2007; Food and Agriculture Organization of the United Nations, 2017). Each *Nannochloropsis* sp inoculum was treated with A, B, C, and D treatments and each treatment was repeated 4 times. Culture was conducted during 7 days. Obervations in this research included the growth of *Nannochloropsis* sp by estimating population density (Fatuchri, 1985), the growth progress (Campoes et.al, 2014), generation time (doubling time) (Pelezar et.al, 1986), and water quality analysis (including DO, pH, temperature, salinity, nitrate, nitrite, ammoniac, and phosphate) which were conducted at the early and the end of culture. The differences of every treatment were analyzed by using *One Way Analysis of Varian* (ANOVA) with significance level of α = 0.05 and then followed by Least Significant Different test (Zar, 1996).

Result and Discussion

The peak of *Nannochloropsis* sp population growth occurred in the fifth day and in the treatment A with highest significant density ($p<0.005$) compared to other treatments with density of 4011.50±626,34 x 10^4 cell/mL (Figure 1).

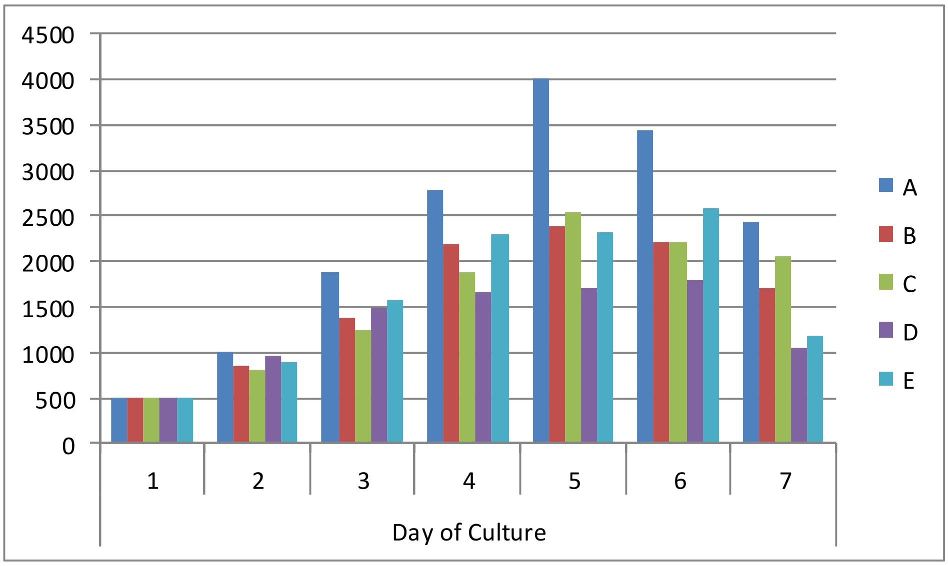

Figure 1. The *Nannochloropsis* sp Density (X 10^4 cell/mL).

The specific growth progress in all treatments showed that in the third day the growth progress started to decrease and the fourth and fifth days were the stationary phase while the sixth day was the death phase. Treatment A showed significant and fastest specific growth rate ($p<0.050$) compared to other treatments (Figure 2).

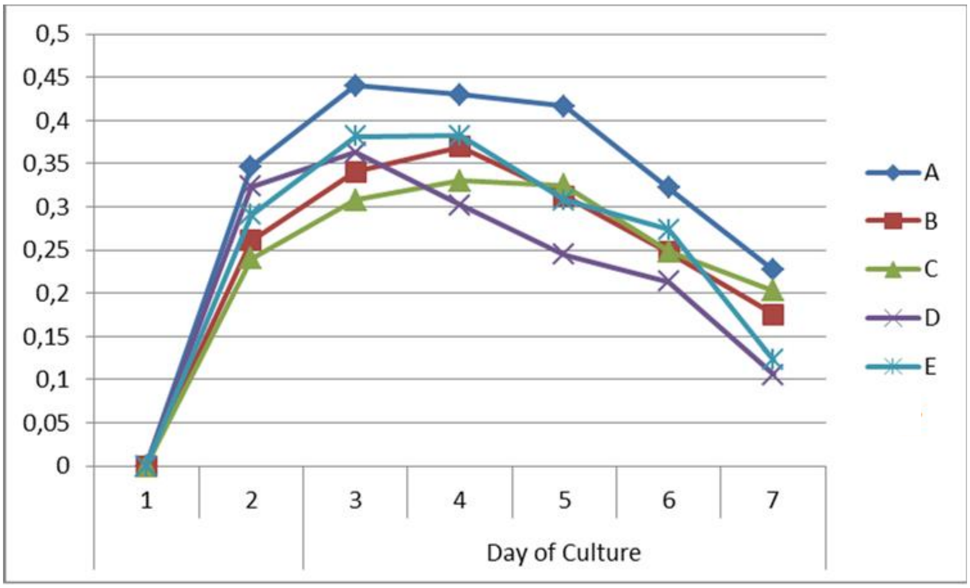

Figure 2. The *Nannochloropsis* sp Specific Growth Rate (cell/mL/day).

The fastest generating time (doubling time) occurred in the third day to the fourth day and started to slow in fifth day. Treatment A showed the fastest generating time (doubling time) significantly ($p<0.050$) to treatment B, D, and E, but it was not different significantly with treatment C ($p=0.065$) (Figure 3).

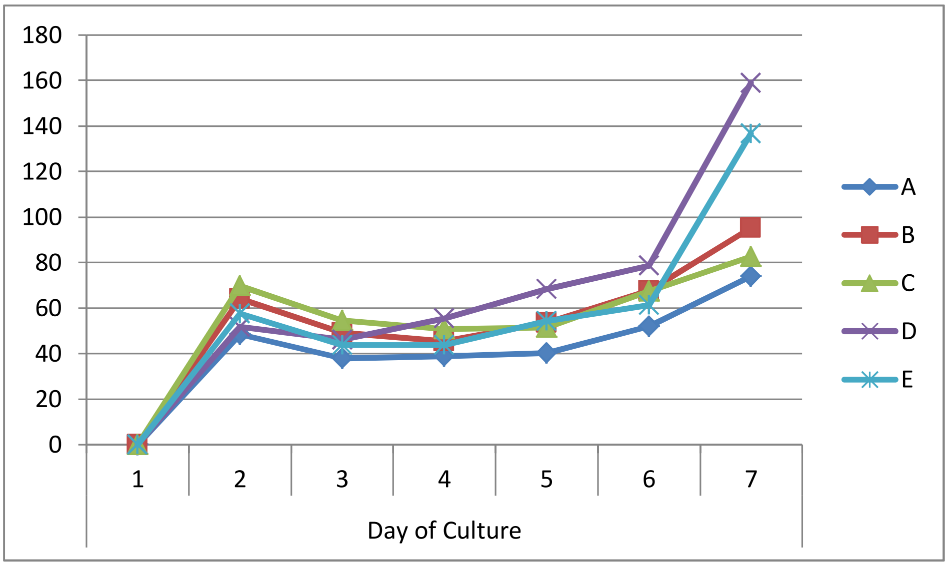

Figure 3. *Nannochloropsis* sp doubling time progress (hour)

Discussion

Microalga requires major and micro nutrients for its growth. The major nutrients are carbon, nitrogen, phosphor and potassium. Sodium bicarbonate or carbon dioxide are carbon sources and the commercial NPK as NPK source. In addition, microalga growth also requires sufficient sunrays, suitable temperature, and optimal combination of NPK optimal (Sivakumar and Rajendran, 2013).

The most important parameter influencing alga growth, physiological activity, and biochemistry composition includes amount and quality of nutrients, light, pH, turbulence, salinity and temperature, and some parameters are mutually dependent where one parameter in a particular condition is a determining factors while the others are less required. Media enrichment has been used in the growth of most alga including Walne, Guillard and Conway (Renaud and Parry, 1994; Coêlho et al., 2013). The growth stage and manipulation of physical and chemical conditions in the culture produce cell composition difference such as variations of protein, fat, carbohydrate contents and other cellular components (Lourenco, 2006). Water ecosystem has very big natural variations of carbon (C), nitrogen (N), and phosphor (P), where its composition is enriched by human activities, and primary productivity is the characteristics of ratios from these element sources (Golz et.al., 2014). Alga has an ability to use CO_2, which is abundant in the sea, as a carbon source in the photosynthesis process (Sirin, et al., 2013; Karemore et al., 2013; Dineshkumar et al., 2015).

The stoichiometric variation of C:N:P composition influence availability of C nutrient ratio to the main producer like phytoplankton (Persson et al., 2010). The main *Nannochloropsis* sp producer growth shows differences significantly in the dependence of element compositions in the nutrient chains in culture media Golz et.al., 2014). C is the important element in the organic macro molecules (carbohydrate, protein, and lipid), while N and P are the important components in specific macro molecules. P component is important in forming RNA, phospholipid and DNA blocks, while N is an important element in forming protein (amino acids) and nucleic acids (Anderson, 2008). Silica is used specially in diatom growth which is used for external shell formation (Hu, 2014; Food and Agriculture Organization of the United Nations, 2017).

The use of agricultural fertilizers in alga culture in outdoor pool is an effort to reduce alga production cost compared to laboratory-grade reagent (Food and Agriculture Organization of the United Nations, 2017). Nitrogen and phosphor concentrations in culture media should be optimized, because nitrogen and phosphor are key nutrients in the microalga growth (Lin and Lin, 2011). The different agricultural fertilizer combinations are used as nitrogen and phosphor sources and micronutrients as potential alternatives to reduce *Nannochloropsis* sp microalga production cost (Lubián, 1982). Some studies has been done to optimize culture media compositions for biomass production and interesting product contents to improve in *Nannochloropsis* sp (Breuer et al., 2012; Griffiths et al., 2012).

Nannochloropsis sp. shows clear reduces in the growth, chlorophyll content, and dry weight, because it is cultivated in the limited nitrogen condition and nitrogen starving. The same condition occurs when *Nannochloropsis* sp is cultured in a treatment of 12:12 hours of dark: light (Alsull and Omar, 2012). *Nannochloropsis* sp can grow in the brackish soil water and even in 2 ppt salinity, and *Nannochloropsis* sp can use urban water waste as single nutrient source in the culture. *Nannochloropsis* sp has economy value because its availability in producing high fat amount which can be used in biodiesel production or other needs as animal feed and aquaculture, human food, biochemistry and pharmaceuticals (Carlsson, 2007; de Sousa, *et al*., 2014).

Conclusion

The highest *Nannochloropsis* sp cellular density from isolate of Lampung Mangrove Center water in intermediate culture scale can be reached in the day 5 and combinations of agricultural fertilizers of urea 40 ppm, Za 20 ppm and TSP 5 ppm are the best for *Nannochloropsis* sp culture in intermediate culture scale.

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