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Original Article

Combination of agriculture fertilizer for intermediate cultivation of isolate *Nannochloropsis* sp. of the waters of Lampung Mangrove Center as live feed

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Abstract

The availability of microalgae (phytoplankton) has an important role in aquaculture. *Nannochloropsis* sp. is a microalgae that can be used as the live feed for larvae cultivation of shrimp, fish and shellfish. In this study, a *Nannochloropsis* sp. isolate obtained from Lampung Mangrove Center waters was cultured by using combination of 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 designed using completely randomized design (CRD), with 5 treatments of agricultural fertilizer consisted of 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: Conway and vitamin B12 1 ppm (control), with 4 replications. The results showed that the treatment of A produced the highest cell density and the fastest growth rate ($p < 0.050$) compared with 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 in significant difference with the treatment of C ($p=0.065$). It was concluded that the combination of the agricultural fertilizer treatment A was 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 (zooplankton and phytoplankton) in normal situation of natural aquatic ecosystem is sufficiently available and even plentiful, and it can be used by each trophic level efficiently, especially fish which belong to the top trophic level. The problem of providing natural feed usually arises when organisms live in cultivation environment. Microalgae have an important function in aquaculture, because the microalgae are the base level of food chain (Lubián, 1982).

Microalgae have an ability to grow fast. They also can produce macro molecules such as lipid, protein, and carbohydrate and chemical material such as unsaturated fatty acids, water-soluble polysaccharides, phycobilins, and carotenoids. For commercial scale, microalgae mass culture were

applied about several years ago and biomass from microalgae have been used for functional of nutraceuticals and food (betacarotene, astaxanthin, and polysaccharides unsaturated). In addition biomass from microalgae were also used as additional feed of marine animal especially aquaculture (as mineral sources and protein) (Hu, 2014; Sivakumar and Rajendran, 2013), source of biofuel (Gill et al., 2016; Chen et al., 2017), material for biocomposite (Zuber et al., 2017) and as heavy metal ion absorption to control water quality in the environment (Buhani et al., 2017; 2017a; 2006; 2012; 2012a; 2011; Buhani and Suharso, 2009).

Microalgae have an important role in aquaculture because it can be applied directly for living feed to larvae stadium of molluscan and crustacean or indirectly as feed for zooplankton species such as nauplii of *Artemia* sp. and *Brachiomus plicatilis* (Barclay and Zeller, 1996). Microalgae especially phytoplankton should have criteria which are able to be used in aquaculture such as non-toxic, having nutrition value, easy to be digested and cultured (Hemaiswarya et al., 2010).

Several microalgae strains are usually used in aquaculture either individually or in combination, based on the nutrition demands. The most common species are *Nannochloropsis* sp, *Scenedesmus* sp, *Isochrysis* sp, *Pavlova* sp, *Dunaliella* sp, *Spirulina phaeodactylum*, *Chlorella* sp, *Rhodomonas* sp, *Tetraselmis* sp, *Skeletonema* sp, and *Thalassiosira* sp (Parrish et al., 2012). *Nannochloropsis* sp is more familiar and known as sea chlorella and it is cultivated in semi-mass or mass scales for zooplankton feed such as *Rotifer* sp *or Barchionus plicatilis* and sea cucumber or shell larvae (Tawfiq et al., 1999; Lubian, 2000).

In the water cultivation (especially hatcheries of shell, fish, and sea cucumber), microalgae culture technique (zooplankton and phytoplankton) are an aspect that must be mastered to support sustainable natural food stock availability. The plankton culture technique in general consists of three stages; the laboratory stage, semi-mass or mass scale, or open pond (Borowitzka and Borowitzka, 1988; Marie et al., 2017).

Nannochloropsis sp is a sea microalgae having high nutrition value, and it is used widely as aquaculture hatchery industry for food of larvae and juvenile of bivalvia, rotifer, as well as fish larvae (Lubián, 1982; Tawfiq et al., 1999). Microalgae 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 result was demonstrated by the high survival rate of *Sparus aurata* larvae being feed with microalgae biomass compared to ordinary feed (Robin and Vincent, 2003).

Nannochloropsis sp is easy to be cultivated in semi-mass and mass scale cultivations as well as it contains non-toxic and antibiotic material (Fulks and Main, 1991). In the culture, the use of pro-analysis fertilizer (Conway) causes very high cost, so that the alternatives of agricultural fertilizers were applied as sources of nitrogen, micronutrients, and phosphate to reduce cost in producing microalgae (Lam and Lee, 2012). Urban wastes were used for *Oedogonium* sp. microalgae culture and it is very potential to produce biocrude oil (Neveux et al., 2016).

The purpose of this study was to determine the combination of agricultural fertilizer use in semi-mass *Nannochloropsis* sp culture in an effort to find the right dose combination of agricultural fertilizer for the growth of *Nannochloropsis* sp.

Material and Methods

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

Treatments	Concentration of Agricultural Fertilizer (ppm)			Conway fertilize as control (ppm)
	Nitrogen (Urea)	Ammonium Sulphate (Za)	Triple Super Phosphate (TSP)	
A	40	20	5	
B	40	20	10	
C	40	20	15	
D	40	20	20	
E	-	-	-	1

Culture of *Nannochloropsis* sp. intermediate scale used a glass aquarium with volume of 100 L. Culture was carried out in a semi out door but it was still protected from direct sun (transparent roof). Inoculum of *Nannochloropsis* sp. from the results of the laboratory scale culture placed in the aquarium as

much as 10% of the volume of the aquarium, each culture medium was given an aeration, and the population density of *Nannochloropsis* sp was calculated every day until harvested. After 4 - 5 days, culture of *Nannochloropsis* sp. was harvested.

The isolate of *Nannochloropsis* sp was collected from Lampung Mangrove Center water ecosystem (Tugiyono 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 (Amini and Syamdidi, 2006). Each *Nannochloropsis* sp inoculum was treated with A, B, C, and D treatments and each treatment was repeated 4 times. The *Nannochloropsis* sp was cultured for 7 days as showed in Fig. 1. Obervations in this research included the growth of *Nannochloropsis* sp by estimating population density, the growth, generation time (doubling time) (Daefi et al., 2017; Sirin et al., 2013; Amini and Syamdidi, 2006), and water quality analysis (including DO (dissolved oxygen), pH, temperature, nitrate, nitrite, ammoniac, and phosphate) which were conducted at the early and the end of culture.

Figure 1. Stage of Nannochloropsis sp intermediate-scale culture (A: laboratory scale culture, B: initial stock propagation for intermediate culture, C: adaptation stage, and D: intermediate scale culture

Results 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 (Fig. 2). In addition, the quality of media culture as an observation results was displayed in Table 2. Concentrations of nitrite (NO_2), nitrate (NO_3), ammonia (NH_3), and phosphate $(PO_4)^{3-}$ in the media culture were around 0.22 - 0.39, 4.51 - 9.30, 2.42 - 2.52, 1.41 - 3.21 mg/L, respectively with temperature of 28 °C and pH of 8.00 - 8.20. These parameters obtained support the growth of phytoplankton especially *Nannochloropsis* sp. for optimum conditions (Creswell, 2010; Gill et al., 2016). The specific growth progress of *Nannochloropsis* sp 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 of A showed significant and fastest specific growth rate of *Nannochloropsis* sp ($p < 0.050$) compared to other treatments (Fig. 3).

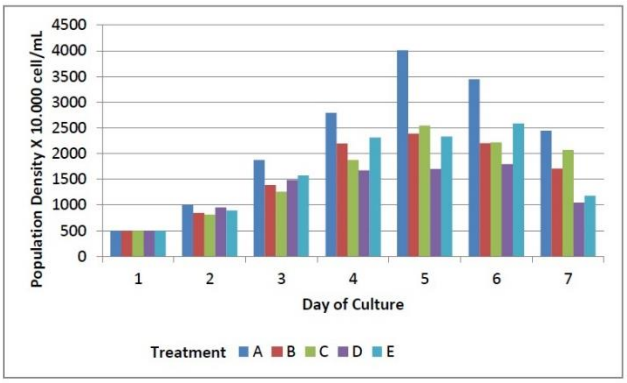

Figure 2: Population Density of *Nannochloropsis* sp.

Table 2: Quality of media culture

No	Parameters	Unit	Observation Results				
			A	B	C	D	E
1	Nitrite (NO_2)	mg/L	0.32	0.22	0.28	0.39	0.28
2	Nitrate (NO_3)	mg/L	6.52	7.02	4.51	9.30	5.04
3	Ammonia (NH_3)	mg/L	2.49	2.52	2.42	2.43	2.47
4	Phosphate	mg/L	1.49	1.41	2.08	3.21	2.01
5	Temperature	°C	30.00	30.00	30.00	30.00	30.00
6	pH		8.10	8.20	8.00	8.10	8.20
7	DO	mg/L	5.50	5.55	5.56	5.53	5.55

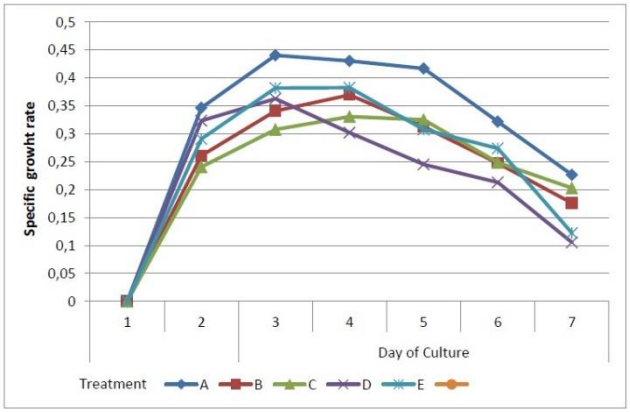

Figure 3: Specific Growth Rate of *Nannochloropsis* sp. Population.

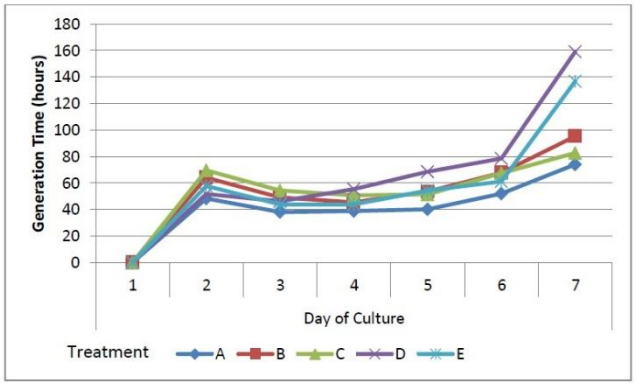

Figure 4: *Nannochloropsis* sp doubling time progress (hour).

Growth phases of plankton consist of 4 phase namely; lag, exponential (logarithmic) growth, stationary, and death (loga rithmic decline) phase (Creswell, 2010). As seen in Fig. 4, the first phase of growth phase of *Nannochloropsis* sp occurred in the first day to the second day as called lag phase. The fastest generating time (doubling time) of *Nannochloropsis* sp started in the second day to the fourth day as called logarithmic growth phase continued with slow growth (stationary growth phase) in the fifth day until the seventh day. Treatment of A showed the significantly fastest generating time (doubling time) of *Nannochloropsis* sp ($p < 0.050$) among treatment of B, D, and E, but it was not different significantly with treatment of C ($p = 0.065$) (Fig. 4).

Microalgae requires major and micro nutrients for its growth. The principal nutrients are nitrogen (N), potassium (K), carbon (C), and phosphorus (P). The source of carbon come from carbon dioxide or sodium bicarbonate and NPK from the commercial NPK fertilizer. In addition, microalgae growth also requires appropriate temperature, adequate sun rays, and optimal combination of NPK (Sivakumar and Rajendran, 2013). Nitrogen contained in urea fertilizer at doses of 50 ppm is a more dominant factor in stimulating Nannochloropsis growth than doses of 40 ppm and 30 ppm in laboratory-scale cultures (Daefi et al., 2017).

Reduction of inorganic nitrate concentration as $NaNO_3$ in culture media tends to inhibit the growth of *Nannochloropsis* sp indicated from a decreasing of growth curve (Muhaemin et al., 2014). Optimum nutrient content is able to spur growth of cell. This leads to better algae population growth, as increasing of algae population growth is always followed by increasing the amount of nutrient to be used to be run out (Round, 1973).

The most important parameters (nitrate (NO_3) and phosphate ($(PO_4)^{3-}$) influencing algae growth, biochemistry composition and physiological activity includes quality and amount of nutrients, pH, light, salinity, turbulence, and temperature as well as some parameters are mutually dependent where one parameter in a particular condition is a determining factors while the others are less required. The pro-analysis fertilizers used as media enrichment in the growth of most algae are the type of fertilizers such as Walne, Guillard and Conway (Renaud and Parry, 1994). The growth stage and physical and chemical treatment in microalgae culture produce differences in cell material composition such as variations in fat, protein, carbohydrate, and other cellular components (Lourenco et al., 2002). Water ecosystems have very wide of natural variations especially in nitrogen (N), carbon (C), and phosphor (P), where their compositions are enriched by human activities and primary productivity of waters (Golz et al., 2015). Algae 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 composition of elements in culture media affects the growth of *Nannochloropsis* sp. as a major producer in the aquatic ecosystem food chain (Golz et al., 2015). C is the important element in the organic macro molecules (lipid, carbohydrate, and protein), 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, 2005). Silica is used especially 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 microalgae 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 microalgae 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 (Rocha et al., 2003; Breuer et al., 2012; Griffiths et al., 2012).

Nannochloropsis sp. shows a significant decrease in growth rate, 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 waste water as single nutrient source in the culture. *Nannochloropsis* sp has economic value because of its ability 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 (Gill et al., 2016; 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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