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EFFECT OF COMBINATIONS OF UREA, ZA, AND TSP ON THE GROWTH RATE AND EXTRACELLULAR POLYSACCHARIDE CONTENT OF *Porphyridium* sp.

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ABSTRACT

The purpose of this study was to determine the provision of combinations of urea, ZA, and TSP to the growth rate and extracellular polysaccharide content of *Porphyridium* sp. The study was conducted using a completely randomized design (CRD) with combination treatment of fertilizer: A (25 mg / l of urea: 30 mg / l ZA: 10 mg / l TSP); B (50 mg / l of urea: 30 mg / l ZA: 10 mg / l TSP); and C (75 mg / l of urea: 30 mg / l ZA: 10 mg / l TSP). Control treatment in the study carried out by using fertilizers conwy and apart from the design above. The parameters observed were the density of population, growth rate, and the content of extracellular polysaccharides.

The data were analyzed using ANOVA at α = 5%. The results of data analysis showed that the combination treatment of fertilizer significantly affected the rate of growth, population density, and the content of extracellular polysaccharide *Porphyridium* sp. Growth rate, population density, and the content of extracellular polysaccharide *Porphyridium* sp., sequentially obtained from a fertilizer with a combination treatment of urea concentration 75 mg/l, 50 mg/l, and 25 mg / l.

Keywords: *Porphyridium* sp., growth rate, and poplation density

INTRODUCTION

Porphyridium is a microalgae that has a native habitat in seawater (Vonshak, 1988). *Porphyridium* produces secondary metabolites are excreted in the form of extracellular polysaccharides through the golgi apparatus into the cell culture medium (Kusumawarni 1998). Extracellular polysaccharide produced by microalgae on a stationary phase serves as a protection of cells from unfavorable environmental conditions (Lee, 2008).Growth and development of microalgae *Porphyridium* influenced by several factors such as temperature,

light, salinity, pH and nutrient content in the culture medium (Vonshak, 1988). Vey (1995) explains that the nutrient is one of the growth determinefactors of microalgae in the culture. According to Brown (1997) conwymedia suitable for the growth and development of microalgae in its culture because conwy media containcomplete elements of macro and micro nutrients. In *Namochloropsis* culture, conwymedia addition of 1 ml/l in the culture produce algal density of 11.08 x 106sel/ml with a cell diameter of 3.19 µm. These results are higher than that which is obtained from microalgae culture containing fertilizers Trace Nutrient Fertilizer (TNF) derived from the decomposition of plant and animal residues. Dose of TNF is 1; 5 and 10 ml/l and each produces the cell density on each peak phase in sequent at 8.33 x 10^6; 10.3 x 10^6; and 5.33 x 10^6sel/ml with cell diameters respectively of 2.18; 3.4 and 3.16 µm (Dayanto *et al*., 2013). The problem of the use of conwy media as a fertilizer or source of nutrients in the cultivation of microalgae is a high price so that the cost burden for farmers to provide fertilizer is very high. Therefore, it needs a fertilizer alternative that costs more affordable, but in accordance with the needs of growing microalgae.

Research on the increase in the growth rate of *Porphyridium* sp. using of agricultural fertilizers has been carried out. Agricultural fertilizer that is used for culturing *Porphyridium* sp.include urea, ZA, and TSP. Nitrogen contained in the fertilizer of urea and ZA also phosphate contained in the TSP fertilizer play a role in increasing the growth rate of microalgae. The addition of nitrogen and ammonium in the culture medium can also increase extracellular polysaccharide content in cultured *Porphyridiumcruentum* (Styaningsih *et al*., 2013). The results of Fogg (1987) indicates that the element of N in the form of nitrate and P in the form of phosphorus are the two main elements that must be present in the culture medium of microalgae. Nitrogen and phosphate nutrients required for the biosynthesis of microalgae protein (Sari *et al*., 2012).

Dose of ZA best in enhancing the growth of *Porphyridium*is 30 mg/l and TSP 10 mg/l. While the dose of urea is most excellent for growing microalgae *Porphyridium* is 50 mg/l (Afriza, 2015). Furthermore Vonshak (1988) states that microalgae, *Porphyridium*, can use KNO3 and ammonium as nitrogen source in its growth.

The purpose of this study was to investigate the combination of concentration of urea, ZA, and TSP which optimal for growth and extracellular polysaccharide content of *Porphyridium* sp.

MATERIALS AND METHODS

Research was conducted in November 2015 until January 2016 at the Laboratory of Aquatic and Botany, Faculty of Mathematics and Basic Sciences, University of Lampung. The tools used are the culture bottles, aerator, paper labels, funnel, plankton-net, aluminum foil, ultraviolet water sterilizer, digital scales, refractometer, pipette, microscope, haemocytometer, measuring cups, cover glass, dark bottle, filter paper , oven, and desiccator.

Materials used are inoculum *Porphyridium* sp. obtained from a stock purely at the Balai Besar pengembangan Budidaya Laut (BBPBL) Lampung which is located in the village of Hanura, TelukPandan, Pesawaran District, Lampung Province; distilled water; alcohol 70%; chlorine; paper towel; sea water; fresh water;media agricultural fertilizer (urea, ZA, TSP), soap; and technical ethanol 96%.

The experiment was conducted using a completely randomized design with 3 treatments. Treatment A (25 mg/l of urea: 30 mg/l ZA: 10 mg/l TSP), treatment B (50 mg/l of urea: 30 mg/l l ZA: 10 mg/l l TSP), and treatment C (75 mg/l of urea: 30 mg/l ZA: 10 mg/l TSP), and each treatment was repeated six times. As a control in this study was the culture of microalgae using the conwy media as a source of nutrition done separately, so it was not included in the analysis of variance and a further test.

Data density of population and the growth rate was analyzed using ANOVA (Analysis of Variance) at α = 5%, while the extracellular polysaccharide content data descriptively explained.

Culture of *Porphyridium* sp.

Culture of *Porphyridium* sp. begins with sterilizing equipment and materials, providing of inoculum and the supply of fertilizers for the treatment. *Porphyridium* inoculum used as much as 1 liter with initial culture density of 150 x 10^6 cells/ml. Inoculum put into culture bottles containing seawater that has been sterilized and fertilized in accordance with the treatment. The calculation of the density of cells under a microscope and using haemositometer done every day for 8 days.

Growth rate

Daily growth rate of microalgae is calculated using the formula:

$$g = \frac{\text{Ln } W_t - \text{Ln } W_0}{t}$$ (Kurniastuty dan Julinasari, 1995)

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g = daily growth rate (cells / mL / day)

t = time (days) or time of W0 to Wt (cells / mL)

W_0 = initial density (cells / mL)

W_t = final density (cells / mL)

Measurement of Extracellular Polysaccharides Content

Measurement of extracellular polysaccharide content of *Porphyridium* sp. done every day for 8 days. A total of 10 mL samples were centrifuged and the supernatant is then taken. Technical ethanol 96% is then added to the supernatant at a ratio of 1: 1. The mixture was then stored in a freezer for 24 hours. Furthermore, the separation of the polysaccharide from the solution by filtration using filter paper Whitmann. The filter paper is then dried in an oven at a temperature of 45 ° C for 6 hours, then weighed (Styaningsih *et al.*, 2013).

RESULTS AND DISCUSSION

The results of Anova at α = 5% indicates that the difference in the concentration of urea from all treatment combinations in this study (A, B, and C) affects the population density of *Porphyridium* sp. significantly starting on the day 4 (Figure 1).

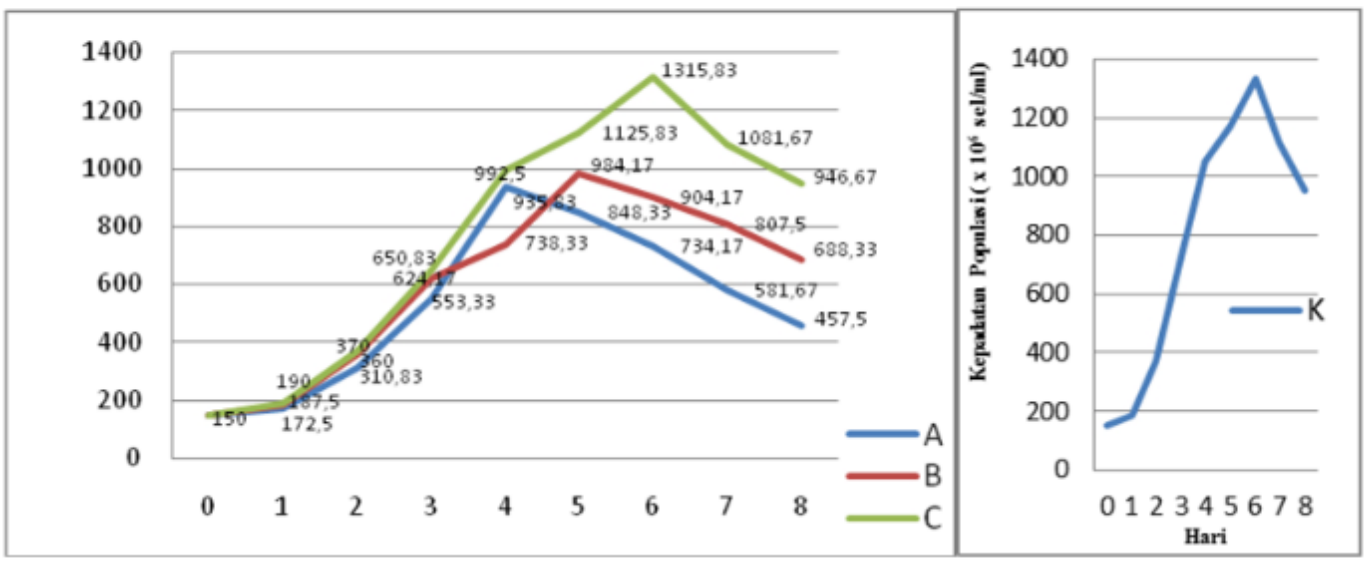

Figure 1. Population density *of Porphyridium*sp. from the age of culture 0 s/d 8 days

Treatment A = 25 mg/l of urea, 30 mg/l ZA, and 10 mg/l TSP

Treatment B = 50 mg/l of urea, 30 mg/l ZA, and 10 mg/l TSP

Treatment C = 75 mg l of urea, 30 mg/l ZA, and 10 mg/l TSP

Controls = fertilizerof conwy 1 ml/l were not included in the analysis of variance and a further test

The fastest exponential phase of the culture *Porphyridium* sp. obtained from the treatment with a urea concentration of 25 mg/l and the slowest obtained from C treatment with urea concentration of 75 mg / l (Figure 1).The data in Figure 1 shows that the lower the nitrogen content in the culture medium of *Porphyridium* sp. causing faster achievement of the exponential phase, but the period of exponential becoming increasingly shorter.The difference in concentration of urea in the culture medium suspected to affect the speed of achievement of the exponential phase and the period time of exponential phase. This presumption is based on the opinion of Herman et al., (2011) which states that the higher the concentration of urea used in microalgae culture medium causes exponential phase lasts longer.The results of this study are also consistent with the results of Ariza (2015) research which showed that in culture of Porphyridium with urea concentration of 10 mg/l, ZA 30 mg/l, and TSP 10 mg/l, the phase of exponential lasts for 4 days while the culture of microalgae with a urea concentration of 50 mg l, ZA 30 mg/l , and TSP 10 mg/l, the exponential phase lasted for eight days. It is suspected that the high nitrogen content in the culture medium causing prolonged cell division resulting in the highest density cells. This presumptionis based onthe results of this studywith the highest concentration of urea is 75 mg/l reached the highest maximum density with exponential phase longest.

The results of the observations for 8 days, indicating that the growth *Porphyridium* sp. obtained from treatment A with a urea concentration of 25 mg/l gave in the lowest number of maximum cell density, while the number of the highest maximum cell density obtained from C treatment with urea concentration 75 mg/l. Subekti *et al*. (2013) found that the higher the concentration of urea used in the culture medium of microalgae led to the rapid growth of the population so that the population number will increase. In *Nannochloropsis* sp. cultures treated with urea fertilizer ratio of 10 g/l, ZA 20 g/l, and TSP 10 g/l on average produce cell number of 1169.66 x 10^4 cells/ml,whereas cultures treated with the ratio of urea 50 g/l, ZA 20 g/, and TSP 10 g/l to produce a cell number 1371.64 x 10^4 cells / ml.

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Table 1. The mean of the *Porphyridium* sp. growth rates as a result of a fertilizer combination treatment

Treatment	**The mean of** *Porphyridium* sp. growth rates**(cells/ml/harday) ±SD**
A	0,123 ± 0,00763 c
B	0,172 ± 0,00918 b
C	0,204 ± 0,00823 a

The number followed by the same letter are not significantly different at LSD, α = 5%

Note: An growth rate mean of the control is 0.205 cells/ml / day

Treatment A = 25 mg/l of urea, 30 mg/l ZA, and 10 mg/l TSP

Treatment B = 50 mg/l of urea, 30 mg/l ZA, and 10 mg/l TSP

Treatment C = 75 mg l of urea, 30 mg/l ZA, and 10 mg/l TSP

According to Herman *et al.* (2011) the growth rate of microalgae is influenced by the media fertilizers used in the culture. While Chrismadha *et al*. (2006) and Widianingsih *et al*. (2008) explains that the nutritional composition in fertilizers that supports the growth of microalgae is the composition of the of fertilizers with a ratio of N: P low. The results of Chrismadha *et al.* (2006) in line with the results of this study, where the rate of growth of phytoplankton or microalgae in culture is limited by the concentration of nitrogen. Nitrogen plays an important role as a constituent amino acids, protein and chlorophyll (Sirappa, 2003).

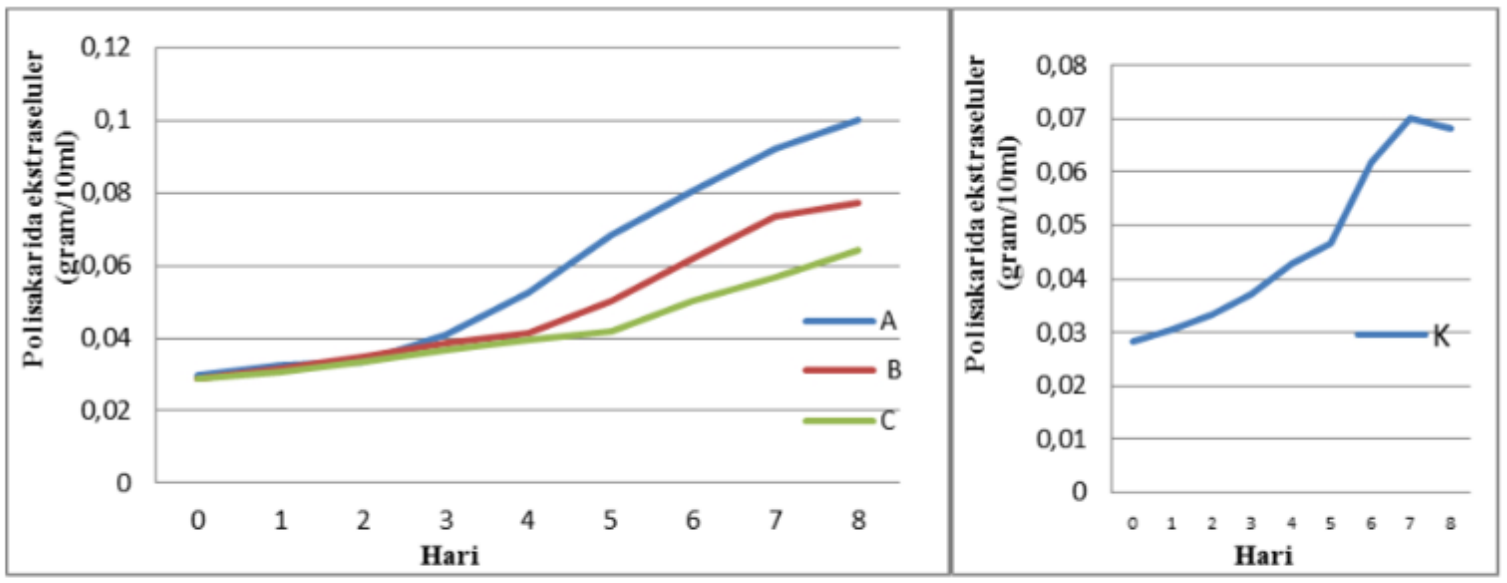

Figure 2. Content of extracellular polysaccharide of *Porphyridium* sp.

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In this study, the highest content of extracellular polysaccharides derived precisely from treatment A and the lowest content of extracellular polysaccharide obtained from the C treatment (Figure 2).

According to Fadillah *et al.* (2014) extracellular polysaccharide production by microalgae takes place during the stationary phase.In the stationary phase microalgae *Porphyridium* sp. adapt to unfavorable conditions, ie when the amount of nutrients in the culture medium decreased, by generating extracellular polysaccharide compound (Styaningsih *et al,* 2013). In this study, the stationary phase of *Porphyridium* sp. growth achieved the most rapid obtained from treatment A, but stationary period of treatment A is the longest. In contrast to the culture *Porphyridium* sp. of treatment C that has a shortest stationary period, the stationary phase is achieved at the latest. Thus the period of the formation and excretion of polysaccharides of treatment A is longer and the extracellular polysaccharide produced becomes more.

Different concentrations of urea in combination of several fertilizers significantly affected the rate of growth *Porphyridium* sp. The concentration of urea best to produce high cell is 75 mg/l, whereas the concentration of urea best to produce extracellular polysaccharides is 25 mg/l.

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