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Research Article

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Effect of Land Agricultural Fertilizer on Growth of Marine Single Cell Protein, *Spirulina platensis, Chlorella vulgaris* and *Nannochlorophsis*

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ABSTRACT

This study aimed to determine the growth rate and protein content in *Nannochloropsis oculata*, *Chlorella vulgaris*, *Spirulina platensis* were cultured using agricultural fertilizers. The agricultural fertilizers used were Urea, ZA and TSP. Each single cell protein was cultured using the three types of fertilizers with a ratio of Urea: ZA and TSP as follows (A) 1: 2: 1 [(10:20:10 g / L)], (B) 2: 2: 1 [(20:20:10 g / L)] and (C) 3: 2: 1 [(30:20:10 g / L)], respectively. The results showed that the best ratio of Urea, ZA and TSP fertilizers to growth of *Spirulina, Nannochloropsis oculata* and *Chlorella vulgaris* was C [30:20:10 (g / L)] treatment. However, phyto-protein content in *Nannochlorophsis* differed significantly between fertilization treatments, C fertilization yielded the highest protein content (28.75±0.05%), when compared with A (25.13±0.01%) and B (25.14±0.02%), respectively. In Chlorella vulgaris, all fertilization treatments showed very significant differences, B fertilization (28.24%±00.006) yielded the highest phyto-protein content, if compared to A (23.63% ± 0.003) and C (19.74% ±0.006), respectively. All fertilization treatments showed very significant differences (P <0.05) on *Spirulina platensis*. The highest content of phyto-protein (62.68 + 0.05%) was present in treatment C, when compared to A (52.18 + 0.05%) and B (62.37 + 0.01%) treatments, respectively.

Keywords: *phyto-protein, growth, fertilizer, marine-algae*

ABSTRAK

Penelitian ini bertujuan untuk mengetahui laju pertumbuhan dan kandungan protein dalam *Nannochloropsis oculata*, *Chlorella vulgaris, Spirulina platensis* yang dibudidayakan menggunakan pupuk pertanian. Pupuk pertanian yang digunakan adalah Urea, ZA dan TSP. Setiap protein sel tunggal dikultur menggunakan tiga jenis pupuk dengan rasio Urea: ZA dan TSP sebagai berikut (A) 1: 2: 1 [(10:20:10 g / L)], (B) 2: 2 : 1 [(20:20:10 g / L)] dan (C) 3: 2: 1 [(30:20:10 g / L)]. Hasil penelitian menunjukkan bahwa rasio terbaik pupuk Urea, ZA dan TSP terhadap pertumbuhan *Spirulina, Nannochloropsis oculata dan Chlorella vulgaris* adalah perlakuan C [30:20:10 (g / L)]. Namun, kandungan phyto-protein dalam Nannochlorophsis berbeda secara signifikan antar perlakuan pemupukan, pemupukan C menghasilkan kandungan protein tertinggi (28,75 ± 0,05%), bila dibandingkan dengan A (25,13 ± 0,01%) dan B (25,14 ± 0,02%). Semua perlakuan pemupukan pada *Chlorella vulgaris*, menunjukkan perbedaan yang sangat signifikan, pemupukan B (28,24% ± 00,006) menghasilkan kandungan phyto-protein tertinggi, masing-masing jika dibandingkan dengan A (23,63% ± 0,003) dan C (19,74% ± 0,006). Semua perlakuan pemupukan menunjukkan perbedaan yang sangat signifikan (P <0,05) pada *Spirulina platensis*. Kandungan tertinggi phyto-protein (62,68 + 0,05%) pada perlakuan pemupukan C, bila dibandingkan dengan A (52,18 + 0,05%) dan B (62,37 + 0,01%).

Kata Kunci : *phyto-protein, pertumbuhan, pemupukan, algae laut*

1. Introduction

Phytoplankton is a photosynthetic microorganism with morphology of cell variants, either single cell or multi-celled, small in size, living in waters, moving very passively, including in prokaryotes or eukaryotes and can grow rapidly with unicellular or multi-cellular structures (Marchetti et al. 2012; Quin, 2011). Phytoplankton is one of the aquatic commodities that have great potential to be utilized properly. Utilization of phytoplankton as a commodity trade or raw materials industry is still relatively limited. The use of single cell proteins from marine phytoplanktons such as *Nannochloropsis oculata, Chlorella vulgaris, and Spirulina platensis*, as a source of nutrients began to attract many interest to practitioners of fish feeding, compared to proteins contained in ground plants, proteins contained in *Nannochloropsis oculata, Chlorella vulgaris, and Spirulina platensis*, higher ie 40% - 80% crude protein on a dry weight basis depending on the species (Wahyudi, 1999; Sukardi and Winanto, 2011,2014). Several types of phytoplankton that have been cultivated and utilized by humans include *Nannochloropsisoculata, Chlorella vulgaris, and Spirulina platensis*, but the productivity of protein content of the three phytoplankton species differ by different species, place and time of maintenance (Chisty, 2007).

Phytoplankton in its growth is closely related to the nutritional factors present in its environment. In general phytoplankton requires nutrients consisting of macro nutrients and micro nutrients. The macro nutrient element consists of 9 N, P, K, C, Si, S, and Ca. Micro nutrient elements consist of Fe, Zn, Cu, Mg, Mo, Co, Mn, and B (Prabowo, 2009; Suminto, 2009). N and P elements are the two most important elements available in phytoplankton culture media. Nitrogen is one of the macronutrients that greatly influence the growth and productivity of phytoplankton biomass because it is needed for protein, fat and chlorophyll formers (Utomo, et al. 2005; Suminto, 2009; Maula, 2010). The purpose of this work was to study the feasibility of single cell protein production using land agriculture fertilizer for marine fitoplankton.

2. Materials and Methods

In the laboratory scale, Conwy PA (Pro analysis) was used, whilst in the semi-mass scale of agricultural fertilizers consisting of Urea, ZA, TSP was applied as a source of nutrients for the growth of *Nannochloropsis oculata, Chlorella vulgaris*, and *Spirulina platensis*. Starter algal culture 50-100 mL in the mid exponential growth phase were used as inoculum giving initial cell densities of the 2.0×10^5 cells mL^{-1} for *Nannochloropsis oculata*, *Chlorella vulgaris* and *Spirulina platensis*. The experiment was conducted at a photoperiod of 12 h low light (3.4 lx) and 12 h dark. The inoculum density calculation was performed at a stationary phase when the inoculum was 4-5 days then for a semi-mass scale culture using semi-indoor treatment. Algal was grown on various fertilizer compositions used for a semi-mass scale i.e. (A) Urea: ZA: TSP 1: 2: 1 with a ratio of 10g: 20g: 10g, respectively, (B) Urea: ZA: TSP 2: 2: 1 with a ratio of 20g: 20g: 10g, respectively; (C) Urea: ZA: TSP 3: 2: 1 with a ratio of 30g: 20g: 10g, respectively. The average of temperature was 23±1 °C in the light period and 20±1 °C in the dark period. All cultures were aerated. Samples (1 mL) of each culture were collected for cell density counts. Calculation of algal cells (six replications per culture) was performed using a *Neubauer hemometerometer*. The specific growth rate (μ) was calculated from the exponential phase obtained through two points, N1 and N2. $\mu = \ln(N2-N1)/(t2-t1)$, where N2 = number of cells mL^{-1} at time of harvest t2 and N1 = number of cells mL-1 at time t1 from day 2 until day 4 prior to stationary growth phase in all three species. Proximate analysis was used to determine the nutritional content such as protein, carbohydrate, fiber and ash content (Takeuchi, 1998).

Data analysis

All data from growth, crude protein, crude lipids were analyzed using two way analysis of variance. The first factor (I) was the type of fertilizer, whilst the second (II) was the marine phytoplankton type. The data were expressed as mean ± SD. The main effect comparation were done using the post-hoc Tukey's, when intraction was not significant. Pair wise comparations were used to determine sinificant different between combinations, if the interaction was not significant.

3. Results and Discussion

In *Nannochlorophsis*, the results showed that in all treatments of treatments A, B, and C had significant increases in cell density. Increased cell density was possible because the nutrients were optimally utilized. A seven-days observation showed that the highest cell density (7987 x 10^4.mL^{-1}) was in treatment C. Cell density peaks at day 5, this phase was referred to as logarithmic phase. It was said to be a logarithmic phase because this phase had cell division characteristics with relatively constant cell growth (Fachrullah, 2011). On days 6 and 7 *Nannochloropsis oculata* underwent a stationary phase, a phase in which growth begins to decline, resulted in decreased cell density. In this stationary phase, factors that limit growth rates were balanced because the ratio of dividing and dead cells was the same number (Isnan Setyo and Kurniastuti,1995).

The results showed that the growth of Chlorella Vulgaris on treatment C experienced very rapid cell division from first to peak phase on d 5 and reached stationary phase on d 7 with value (8375 x 104.mL-1). In the C treatment, the peak phase until the stationary phase also decreased drastically due to the growth of Chlorella Vulgaris cells in more culture medium, but the amount of nutrient content in the culture medium decreased so that Chlorella cells gradually died. Stationary stage occurred because the cell death rate was higher than the cell growth rate so the population density decreases (Yusi, et al. 2012). Urea was the largest contributor of ammonium ions in the growth medium compared to ZA and TSP, resulted in growth of Chlorella Vulgaris very quickly. Nitrogen played an important role in cell growth, cell lengthening and cell differentiation stages showed that urea acted as the largest contributor of ammonium ions in the growth medium compared to ZA and TSP (Prabowo, 2009).

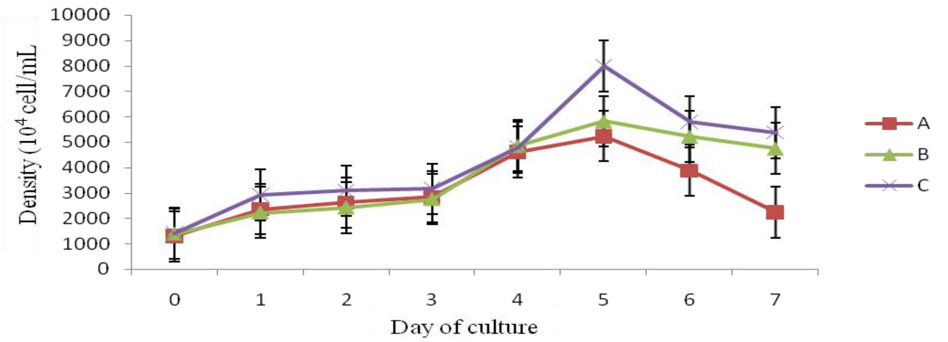

Figure 1. The growth of *Nannochloropsis oculata* (mean±SD), (A) Urea : ZA : TSP (1 : 2 : 1) with ratio 10g : 20g : 10g, (B)Urea : ZA : TSP (2 : 2 :1) dengan rasio 20g : 20g : 10g, (C) Urea : ZA : TSP(3 : 2 : 1) 30g : 20g : 10g.

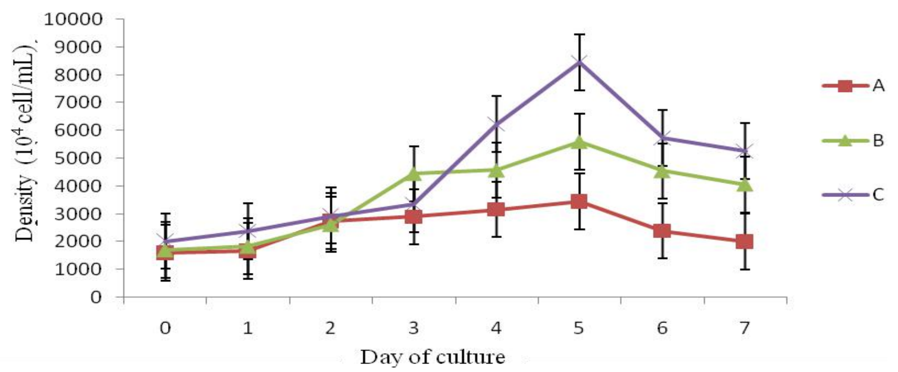

Figure 2. The growth of Chlorella vulgaris (mean±SD), (A) Urea : ZA : TSP (1 : 2 : 1) with ratio of 10g : 20g : 10g, (B)Urea : ZA : TSP (2 : 2 :1) 20g : 20g : 10g, and (C) Urea : ZA : TSP(3 : 2 : 1) 30g : 20g : 10g, respectively.

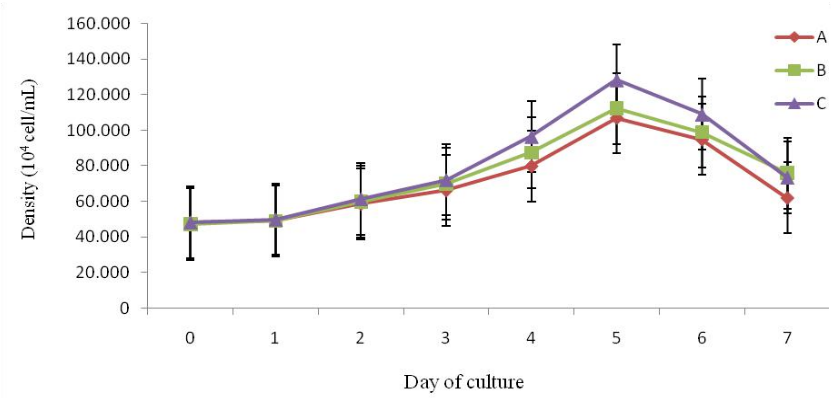

Figure 3. The growth of *Spirulina platensis* (mean±SD), (A) Urea : ZA : TSP (1 : 2 : 1) with ratio of 10g : 20g : 10g, (B)Urea : ZA : TSP (2 : 2 :1) 20g : 20g : 10g, and (C) Urea : ZA : TSP(3 : 2 : 1) 30g : 20g : 10g, respectively.

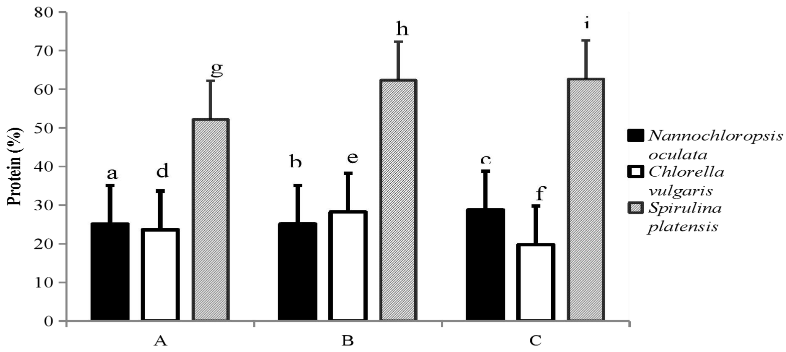

Figure 4. The phytoprotein content of *Nannochloropsis oculata, Chlorella vulgaris* and *Spirulina platensis* (mean±SD) using concentrations of fertilizers (A), (B) and (C), respectively (P<0.05)

It can be seen in Fig. 3 that *Spirulina platensis* growth on day 2 to 5 was increased and peak phase occurred on d 5 (1283,3x 10^4). At the beginning of the culture that occurre phase of adaptation, day 2 incremented up to 5. On day 5 all treatments occurred peak phase or increased density, the change was due to the occurrence of optimal cell division. Then all the treatment underwent stationary phase on days 6 and 7. Stationary phase where in this phase of growth begun to decrease, so the cell density decreased (Widiyaningsih, 2008).

The results in the treatments A showed that phytoprotein content in *Spirulina platensis, Nannochloropsis oculata* and *Chlorella vulgaris* were 52.18%, 25.13% and 23.63%, respectively. In B treatment the phyto-protein content of *Spirulina platensis, Chlorella vulgaris* and *Nannochloropsis oculata* were 62.37%, 28.24% and 25.14%, respectively, whilst in the treatment C, *spirulina, Nanno* and *Chlorella* were 62.68%, 28.75% and 19.74%, respectively. In all treatments fertilization showed that spirulina platensis had higher phyto-protein content when compared to *Nanno chlorophsis* and *Chlorella vulgaris. Spirulina platensis* had the highest protein content in the C treatment of 62.68%. It indicated that the nutrients contained in the culture medium had been in accordance with the needs of *Spirulina platensis* nutrients which were in agreement with Amanati et al. (2013) study using urea fertilizer for a marine single cell protein. Phytoplankton grew in sufficient conditions of nitrogen during culture tends to accumulate biomass and protein (Noorun, et al. 2014).

4. Conclusions

Marine phytoplankton grew well using agricultural fertilizer, the best fertilizer ratio was 30g urea, 20g ZA, and 10g TSP, respectively. The highest production was *Spirulina* (128334x 10^4.mL^{-1}), *Chlorella* (8375 x 104.mL-1), and *Nannochloropsis* (7987 x 10^4.mL^{-1}), respectively. The protein content of *Spirulina platensis, Nannochloropsis oculata* and *Chlorella vulgaris* was 62.68%, 28.75% dan19,74%, respectively.

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