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Strategy of *Nannochloropsis* Against Environment Starvation: Population Density and Crude Lipid Contents

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ABSTRACT

Nannochloropsis sp., an unicellular marine microalgae, has potential function as a food source for fish larvae and in chemical industry. Microenvironmental conditions, especially nitrogen and salinity stress in marine ecosystems, became major factor affecting the growth of Nannochloropsis sp.The aim of the experiment was to study effect of different salinities and nitrogen dosages to the growth and lipid contents of Nannochloropsis sp. cells.The experiment was arranged in factorial with three replicates. The first factor was salinity (28 ppt and 38 ppt), and the second factor was nitrogen dosage (100% and 50%). Results of the experiments indicated that nitrogen starvation and high saline concentration affected cell density of Nannochloropsis sp. in different level. The results showed that combination treatments of nitrogen starvation and salinity reduced cell density (treatmant A, B, and D), except in treatment C. Combination of high salinity and high N dosages resulted ini steady growth of Nannochloropsis sp. These results suggest that Nannochloropsis sp. are able to overcome salinity stress (38 ppt) in the presence of optimum concentration of nitrogen in the growth cultures.The results also shows that there were no significant differences of crude lipid content between treatments and no correlation between population density and crude lipid content. These results suggest that no simple correlation between population density and crude lipid concentration. On the other hand, population density was not the only factor affected crude lipid concentration in the microalgae cell.

Keyword: Nannochloropsis, population, crude lipid

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I. INTODUCTION

*Nannochloropsis*sp.is anunicellular marine microalgae that can be considered as an important source of industrial fine chemicals (PUFAs, amino acids etc.) and food fishlarvae (Borowitzka, 1995; Kay, 1991). The microalgae nutritional source became potential economically (Otles and Pire, 2001). In addition, the microalgae grown in normal condition may use only for food fish larvaepurposes. For industrial purposes, the rapid and higher production of renewable sources and fine chemical compounds should be determined by manipulated environmental conditions (Romano *et al*, 2000).

Researches may show micro environmental stress affect microalgae internal systems. Muhaemin (2011) showed that short expose to environment stresses on *Nannochloropsis*sp.caused the fluctuation on lipid production. Al Hasan*et al* (1987) and Hanaa*et al* (2004) showed positive correlation between changes on growth, pigmentation, and lipid composition of *Dunaliellasalina*.

The survival ability of microalgae depends on its adaptation and responds to new ecological stresses. Nutrient starvation and salinity are the most habitual stresses in many marine environment systems (Muhaemin, 2010). Both, eukaryotic and prokaryotic microalgae have to solve these stresses to maintain their metabolic system properly. Two strategies related to microalgae's survival ability are as follows(1) low metabolic rate with the rapid full metabolism and cell division as soon as nutrients are available in ecosystem; and (2) shutting off almost all cell metabolic activities (called *cryptobiosis*) (Hanhua and Kunshan, 2006). Further research should be conducted to find which initial strategy reflected by *Nannochloropsis*sp. as an adaptation or respond to micro environmentalstress as defined.

II. MATERIALS AND METHODS

The research was conducted in The Laboratory of Aquaculture University of Lampung, starting from October to November2012. Pure isolates of *Nannochloropsis*sp.were selected as experimental materials in this study. The algal species was obtained from The Laboratory ofMarine Culture Developing Centre at HanuraLampung. The algae was grown in batch cultures under the laboratory conditions as describe elsewhere (Muhaemin, 2008).*Nanochloropsys*sp.was cultured in a 5 L flask containing 4 L culture medium of NaCl (28 and 38 ppt) and nitrogen (100 and 50%). The Conway composition was used to culture media of *Nannochloropsis*sp.The batch cultures were illuminated continuously with fluorescent lamp (Philips 40 W) and light intensity level was *ca.* 200 $W.m^{-2}$.

The microalgae cell were harvested by centrifugation at 5000 rpm for 10 min and stored in a freezer at -20^0C. The dry weight method and optical density were used to measure the growth of *Nannochloropsis*sp. during the experiment (Muhaemin, 2008).

The modified lipid extraction method as described by Hanaa*et al* (2004) and Xu*et al* (1998) was used in the experiment to determine lipid content of *Nannochloropsis*sp. The microalgae cells were extracted with mixture of distilled water, chloroform, and methanol (8:10:20 v/v/v), twice and sonicated for 10 min. The sonicated cells were filtered on to 47 μm diameter GF/C Whatman glass microfiber filters. Chloroform (10 ml) and distilled water (10 ml) were added subsequentially to the filtrate and sonicated for 10 min. The resultant solution was filtered under vacuum through a 25 mm diameter Whatman glass filter microfiber. The filtrate was washed in 30 ml of 5 % NaCl solution, and then the lower layer of $CHCl_3$ was separated and dried over anhydrous sodium sulfate. The solvent was removed through evaporation at 40^0C under reduced pressure. The total lipids were weighed and stored at -20^0C until analysis.

The data were analyzed by χ^2-test (α=0.05) to identify significant differences between treatments. All analysis performed using SPSS ver. 15.

III. RESULTS AND DISCUSSIONS

Figure 1 showed the population density (cell density) of *Nannochloropsis*sp.for all treatments. The cell density fluctuated between times for all treatments. In the other hand, it showed that all treatments tend to have the same population density trend, approximately. They described at least the increasing and decreasing cell density phenomena which might vary between times for all treatments.

All Treatments (A, B, C, and D) have the same trend in DOC-1 to DOC-3. They tend to increased (DOC-1 to DOC-2) and decreased (DOC-2 to DOC-3). All Treatments (A, B, C, and D) the different phenomena in DOC-3 to DOC-5. Cell density in treatment B and D decreasedslowly, although treatment A tends to decreased slightly. In treatment B, the cell density showed slightly different increase compared to other treatments. Muhaemin (2009) showed that fluctuation of cell density highly correlated with the population adaptation of microalgae under environmental stress.

The cell density fluctuation of *Nannocholoropsis*sp. during the experiments showed the existence of adaptation mechanisms triggered by initial treatments of salinity and nitrogen starvation.Generally, nitrogen starvation and high saline condition may affect cell density in different level. The results showed that combination treatments of nitrogen starvation and salinity reduced cell density (treatmant A, B, and D), except in treatment C. Combination of high salinity and high N dosages resulted ini steady growth of *Nannochloropsis*sp. These results suggest that *Nannochloropsis*sp. are able to overcome salinity stress (38 ppt) in the presence of optimum concentration of nitrogen in the growth cultures.

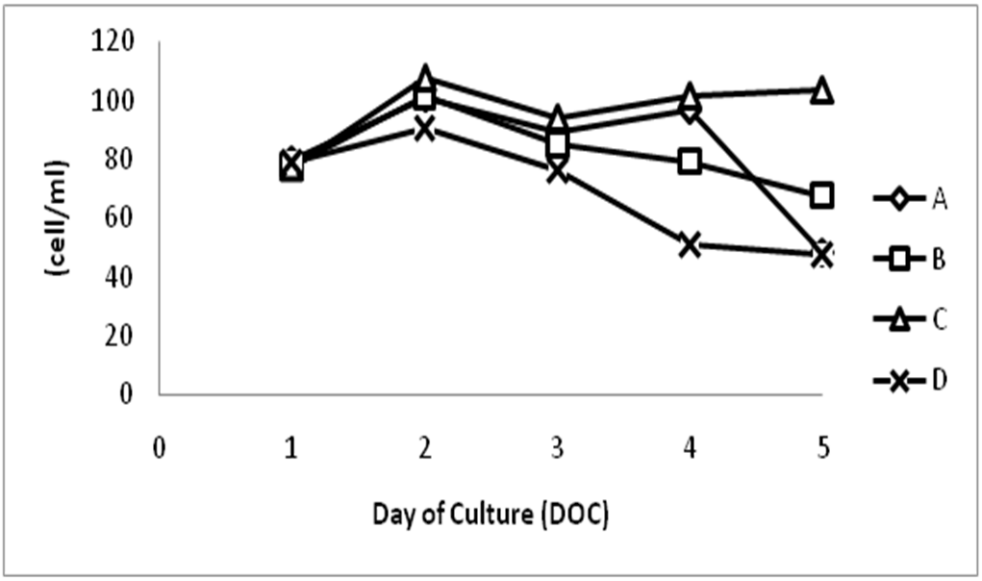

Figure 1.Effect of nitrogen starvation and salinity stress on population density of *Nannochloropsis* sp. (x10^{6}cell/ml). A = Salinity 28 ppt and N dosage 100%; B:salinity 28 ppt and N dosage 50%; C: salinity 38% and N dosage 100%; D: salinity 38% and N dosage 50%.

Based on χ^2 (chi-square) test, (1) there were **no significant** differences on cell density in DOC-1 (early culture stage) for all treatments; (2) there were **no significant** differences on cell density in DOC-5 (end culture stage) for all treatments; and (3) there were **significant** differences on cell density between DOC-1 (early culture stage) to DOC-5 (end culture stage) for all treatments. Theseall phenomena showed that (1)no significant different condition between all treatments in the first stage of culture (the culture condition was

homogenous in the early culture stage) and so did in the last culture stage; (2) the microalgae *Nannochloropsis*sp. has different initial respond by fluctuating their population growth during the experiments.

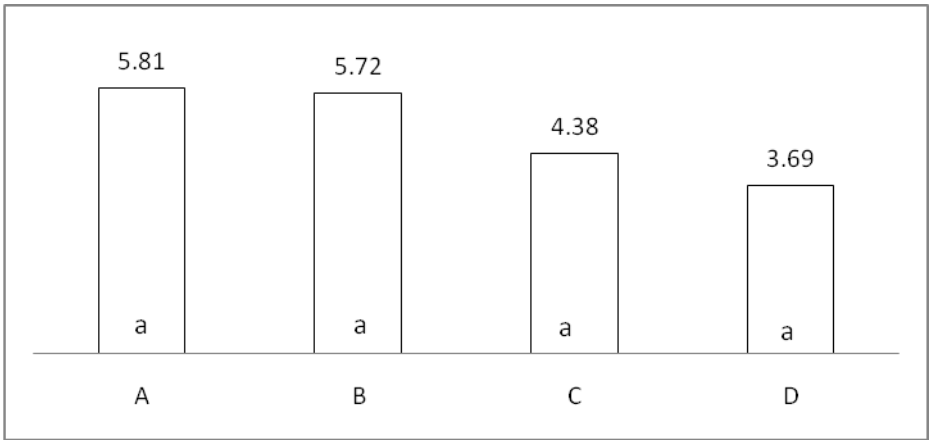

Figure 2. Crude lipid concentration in *Nannochloropsis*sp. cell.

Capital letters describe treatments. Small letters describe no significant differences between treatments based on χ^2 test(α=0.05).). A = Salinity 28 ppt and N dosage 100%; B: salinity 28 ppt and N dosage 50%; C: salinity 38% and N dosage 100%; D: salinity 38% and N dosage 50%.

Figure 2 described the concentration of crude lipid in *Nannochloropsis*sp. cell in the end of culture stage (DOC-5). The A treatment showed the highest concentration (5.81 %) and D treatment showed the lowest concentration.Pratoomyot*et al.* (2005) described that lipid (fatty acid) concentration in marine microalgae was quietly depend on light intensity, salinity, and temperature of culture medium. Otles and Pire (2001) showed that it might vary during the microalgae growth stages. The statistical test showed that no significant difference of crude lipid concentration between treatments.

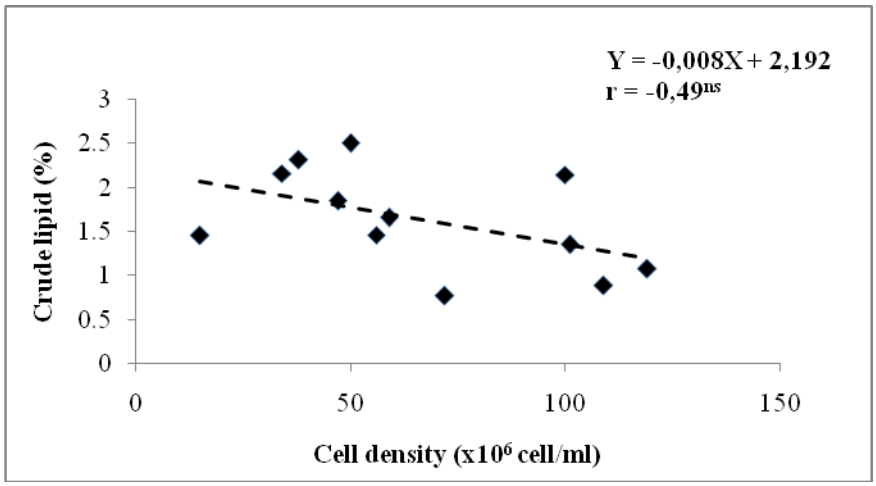

Figure 3. The linier correlation between population density and crude lipid contentin the cells of *Nannochloropsis*sp. Y=crude lipid content (%); X=cell density (cell/ml); R^2=deterministic coefficient; r=correlation ratio.

Figure 3 described the linier correlation between population density and crude lipid concentration in *Nannochloropsis*sp.cells. Generally, the equation {Y=-0.008X+2.192} and correlation coefficient {r=-0.49} showed the same phenomena. Both defined the negative correlation between cell density and crude lipid concentration or that the crude lipid concentration tended to decrease by the increasing of cell density. Muhaemin (2011) showed that the low correlation coefficient indicated no simple correlation between population density and crude lipid concentration. On the other hand, population density was not the only factor affected crude lipid concentration in the microalgae cell.

IV. CONCLUSION

Nitrogen starvation and salinity have different effectsonthe population density of *Nannochloropsis*sp.In the combination of high nutrient starvation and high salinity, the cell density of *Nannochloropsis* sp. washighly depleted. Crude lipid concentration varied and no significant correlation with cell density of *Nannochloropsis*sp.Nitrogen starvation and salinity stress only affected to population density but not to crude lipid content.

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