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Comparison of Growth Performance of Nannochloropsis oculata Biofilm at Different Layers of Substrate Plastic

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Abstract: Biofilm based microalga is a structured community of microalgae attaching to substrate with a self-produced matrix of extracellular polymeric substances (EPS). One of microalgae that is potential to be improved as biofilm is *Nannochloropsis oculata*. Biofilm of *Nannochloropsis oculata* has some beneficial roles such as bioremediator of polluted waters. This study was aimed to evaluate the growth performance of *Nannochloropsis oculata* at different layers of substrate plastic as biofilm substrate. The experimental design used was Completely Randomized Design (CRD) with four level treatments and three replications. Several treatments tested were A: Control (without layer), B: 1 Layer of substrate, C: 2 Layers of substrate, D: 3 Layers of substrate. Several procedures were conducted in this study which were preparation of KW21 medium, cultivation of biofilm at different teratments in KW21 medium, and growth measurement. The results of this study showed that different number of substrate layers could affect the specific growth rate of free cells of *Nannochloropsis oculata*. The highest growth rate was found in treatment B (1 substrate layer) and the lowest one was in treatment D with their values 0.064 μ/day and 0,037 μ/day. The peak population of *Nannochloropsis oculata* was obtained at day 14 until 15.

Keywords: Biofilm; Growth Rate; *Nannochloropsis oculata*; Plastic

1. Introduction

Microalgae are microorganisms that, according to their adaptive capabilities, are able to absorb inorganic materials. In contaminated conditions, microalgae respond by generating phytohormones and polyamines [1]. Because of their capacity for adaptability and absorption, microalgae have the potential to be used as bioremediation agents in contaminated environments. One kind of microalgae that may be used as a bioremediator is *Nannochloropsis oculata*.

Nannochloropsis oculata grows really quickly [2]. At various N:P ratios, *Nannochloropsis oculata* grows at the quickest rate, reaching 135x10^4 cells/ml/day. [3] Undoubtedly, rapid growth can result in the production of a sizeable amount of biomass. To encourage the huge biomass of *Nannochloropsis oculata*, formation of free cells into biofilm becomes one alternative that is appropriate. The process of biofilm formation uses extracellular polymers (EPS) to produce a dense coating of microalgal cells [4]. *Nannochloropsis oculata*'s capacity to build biofilms is anticipated to be a possibly developed remediation agent.

Usage of plastic substrate to help biofilm formation is a technology found by researchers. According to [5], substrate characteristics namely texture, roughness, hydrophobicity then the colonization period and site become determination factors in biofilm formation. However, the influence of different layers of plastic substrate is still unknown and becomes novelty of this

research. This study is aimed to evaluate the effect of different layers of plastic substrate to growth performance of *Nannochloropsis oculata*.

2. Materials and Methods

Materials used in this experiment included seawater, fertilizer K21, mica plastic as a biofilm substrate, and the inoculant *Nannochloropsis oculata*. The study also made use of a few pieces of laboratory equipment, including analytical balance, microscope, and haemacytometer.

a. Experimental design

A laboratory experimental approach with a completely randomized design was employed in the study. Different numbers of biofilm layers applied to KW21 medium were the single factor in this study. There were four levels of treatment, then each level was replicated three times. The treatment levels were: A. control (no substrate), B. one layer of substrate, C. two layers of substrate, D. three layers of substrate.

b. Preparation of modified KW21 medium

A standard medium of KW21 fertilizer was prepared by adding fertilizer KW21 at a concentration of 1ml/L saltwater (30 ppt). Then, sodium bicarbonate (NaHCO3) was added into the medium at a rate 0.25 grams per liter. The medium was designed to hold three liters each container.

c. Cultivation of biofilm in modified KW21 medium

Cultivation was initiated by prepared substrate layer. The layer was 10x10 cm^2 of plastic which served as a substrate for the *Nannochloropsis oculata* biofilm to form. Then, containers that were filled by KW21 medium, were separated into several groups. There were nine containers used in this study, three of which held one layer each, three of which held two layers each, and three of which held three layers each. Then, 20% of the medium volume was filled with the *Nannochloropsis oculata* inoculant. After 17 days of cultivation, the substrate's surface was covered in biofilm. The cultivation was conducted under lighting using a 20 watt of TL Lamp (2000 lux intensity) and aeration for 24 hours [6]. The growth of free cells of *Nannochloropsis oculata* were measured during the study.

d. Growth measurement

Growth measurement was conducted by observing cell density of free cells per day during cultivation of biofilm. Observation used microscope with magnification….

e. Data analysis

Analysis of Variance (ANOVA) for Completely Randomized Design was used to analyze the data of this study. Then, Least Significant Different (LSD), was used to continue the data analysis if there was a significant effect. Significance threshold 95% was employed in data analysis that was helped by software SPSS 21.

3. Results and Discussion

a. Growth of free cells in biofilm formation of Nannochloropsis oculata

Based on the research results, free cells of *Nannochloropsis oculata* in modified KW21 medium with different substrate layer treatments showed different daily densities and population peak. The daily densities of several treatments in this study is shown by Figure 1 below.

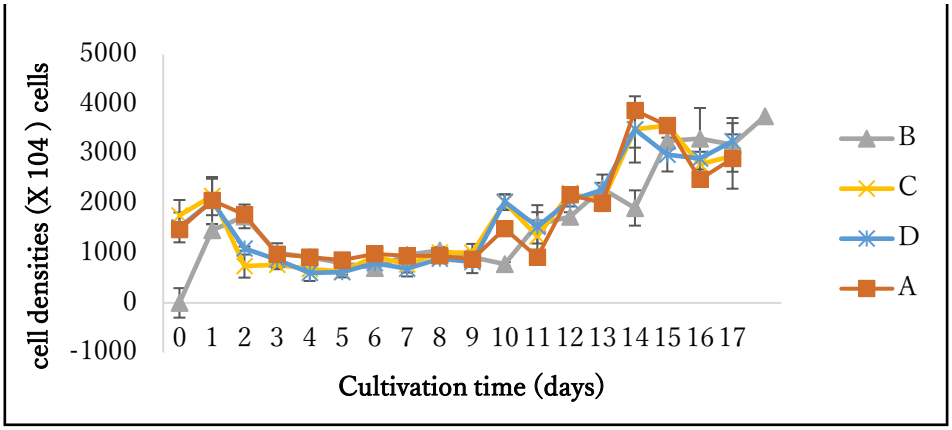

Figure 1. Free Cell Density of *Nannochloropsis oculata* at various substrate layer in KW21 medium (A. control, B. 1 substrate layer, C. 2 substrate layers, D. 3 substrate layers)

Nannochloropsis oculata cells cultured in this study experienced the exponential phase on days 10-14 across all treatments. After the adaptation period ended on 9th day, accelerated growth occurred during the exponential phase. The exponential phase is the phase where microalgae will experience rapid growth, characterized by a very fast increase in cell numbers through the division of microalgal cells [7].

The death phase of *Nannochloropsis oculata* in this study began on day 16. The death phase is characterized by a continuous decrease in density, as the nutrients in culture medium have been well utilized in the previous phase. The death phase is a decrease in the number of cultured organisms after passing through the stationary phase [7][8].

Furthermore, the highest growth rate of *Nannochloropsis oculata* was obtained in treatment B (1 substrate layer), meanwhile the lowest growth rate was obtained in treatment D (3 substrate layers). The application of different numbers of substrate layer affected ($p<0.05$) the specific growth rate of *Nannochloropsis oculata*. The distribution of the specific growth rate for each tretament is shown in the following Figure 2.

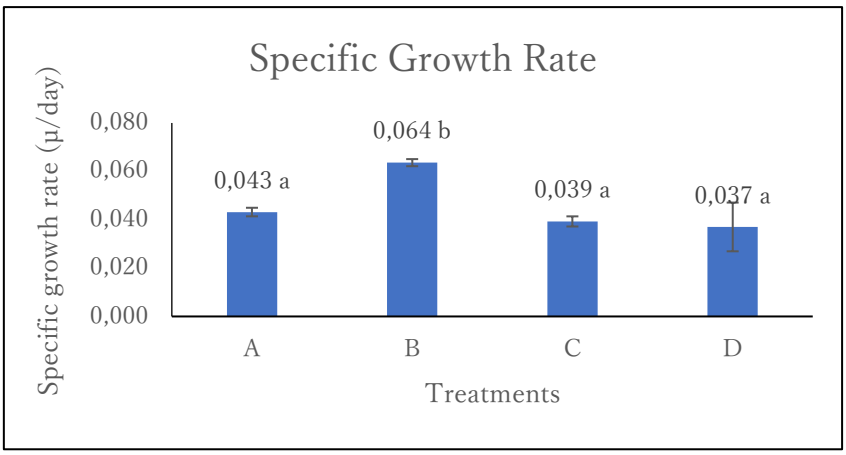

Figure 2. The Specific Growth Rate of Nannochloropsis oculata at Different Substrate Layers

The existence of plastic substrate led free cells of *Nannochloropsis oculata* to attached and formed thin layer of biofilm. The inherent ability of cells influenced the number of *N. oculata*'s free cells. Then, attached cells preferred to produce extracellular polymeric substances (EPS) than reproduce new cells to generate the density. [9] The degree of assortment that forms during biofilm growth is significantly increased by higher dilution levels and thus lower founder cell densities.

4. Conclusions

The different number of substrate layers could affect the specific growth rate of free cells of *Nannochloropsis oculata*. The highest growth rate was found in treatment B (1 substrate layer) and the lowest one was in treatment D (3 substrate layers) with their values 0.064 μ/day and 0,037 μ/day. The peak population of *Nannochloropsis oculata* was obtained at day 14 until 15.

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