

Effectiveness of Algae Growth Promoter on Specific Growth Rate and Growth Phase of *Chaetoceros calcitrans*

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Abstract. This research is motivated by efforts to provide high-quality microalgae as natural feed for shrimp hatchery activities. Researchers have used fertilizers with appropriate N/P ratios, including commercial fertilizers. In this study, the author examines the use of the commercial fertilizer Algae Growth Promoter (AGP) as fertilizer in *C. calcitrans* culture. Current fertilizer use still largely focuses on specific growth rate and increasing microalgal density, which is considered fast, and pays less attention to microalgal nutritional content. The study administered AGP fertilizer (80, 90, 100, 110, 120 ppm) and a control treatment, with 100 ppm modified Guillard fertilizer added, over a culture period from the exponential to the stationary phase. AGP fertilizer reached the stationary phase later, at the 108th observation hour. This is superior to the control treatment, which entered the stationary phase at 72 hours of culture. The control treatment reached the exponential phase earlier, with a td value of 20,29 hours, the shortest doubling time among all treatments. Observations of growth rates were also carried out, where the control treatment was found to have the best specific growth rate, with a μ value of 0,0343 per hour, while the treatment with AGP fertilizer had a specific growth rate with a μ value ranging from 0,0255 to 0,0279 per hour, and the td value ranged between 24,89 and 27,27 hours.

Keywords. Cell Density; *Chaetoceros calcitrans*; Doubling Time; Fertilizer; Specific Growth Rate

I. INTRODUCTION

Chaetoceros calcitrans is a microalgal species widely cultivated in shrimp hatcheries because of its high nutritional content, relatively easy cultivation, and size that matches larval mouth openings. *C. calcitrans* contains 43.10% protein, 11.71% fat, chlorophyll-*a* at 2.4174 mg/L, 19.3% EPA, 0.7% DHA, 21.5% Omega-3, and 38% PUFA, all of which are required by larvae during the early growth phase [1][2][3]. Carotenoid pigments also provide physiological benefits related to adapting to environmental conditions in the rearing medium [4].

Meeting the need for high-quality natural feed is an important prerequisite for an efficient, sustainable shrimp hatchery system aimed at increasing the productivity of the

national shrimp farming industry [5]. argues that the larval phase is the most crucial stage because it determines survival rates and growth performance during the subsequent grow-out phase. Unfortunately, nutrient synthesis in *C. calcitrans* requires sufficient nutrients and an optimal growth period. If these requirements are not met, nutrient formation in *C. calcitrans* may not proceed optimally, and the microalgae may already be entering the death phase. Many factors influence this process, ranging from unstable environmental conditions to insufficient nutrient availability for microalgal metabolism.

Chaetoceros calcitrans cultivation generally uses Guillard medium (f/2 medium) as the primary nutrient source. This medium contains macro- and micronutrients that support microalgal growth on both laboratory and production scales. Productivity in mass-scale cultures

often varies because of environmental factors, nutrient proportions, and media stability [6]. These conditions require alternative media formulations that maintain consistent, efficient microalgal growth across various culture conditions. A commercial fertilizer marketed under the trade name Algae Growth Promoter (AGP) was developed to meet this need. This product contains macronutrients such as nitrogen at 3610 mg/100g, phosphorus (426.55 mg/100g), and potassium (1443.84 mg/100g), as well as micronutrients including magnesium (36.68 mg/100g), calcium (3.46 mg/100g), manganese (412.28 mg/100g) and iron (451.87 mg/100g) of these nutrients play an important role in supporting cell growth and photosynthesis compared to the modified Guillard fertilizer commonly used by hatcheries [7].

Previous research shows that fertilizer type and concentration affect the growth and biochemical composition of the microalga *Chaetoceros* sp. Increasing the KNO₃ dose in Guillard fertilizer accelerated entry into the exponential phase, although it did not significantly affect dry biomass or the growth rate of *C. calcitrans*. The 75 g/L KNO₃ treatment resulted in higher protein and amino acid contents than the other treatments [8]. Guillard and Walne media did not show significant differences ($P>0.05$) in the specific growth constant of *C. gracilis*, but protein and omega-3 fatty acid EPA content were higher in Guillard media than in Walne media [9]. These results confirm that nutrient composition in the culture medium plays an important role in determining growth efficiency and biomass quality, providing the basis for the research objective of analyzing AGP's ability to increase cell density and specific growth rate in *C. calcitrans*.

II. METHODS

2.1. Time and Place of Research

This research was conducted in April 2026 at the Natural Feed Laboratory of PT. Centralpertiwi Bahari Hatchery, Situbondo, for culturing and observing *C. calcitrans* growth. We analyzed the fertilizer's nitrogen and phosphorus content at PT. Saraswanti Indo Genetech Laboratory.

2.2. Experimental Design

This study used a completely randomized experimental design. The test material was a *C. calcitrans* inoculum with an initial density of $\pm 10 \times 10^4$ cells/ml, and the culture medium salinity was 33 ppt. The inoculum came from the Live Feed Laboratory of PT Centralpertiwi Bahari (Situbondo Hatchery) and was pre-cultured with the respective test fertilizers to avoid inconsistent results. This

study included 6 treatments, each replicated 3 times, for a total of 18 experimental units.

We conducted the research indoors using 15-liter gallons, maintaining the room temperature at 18-19°C with continuous aeration and bright lighting at 3000-5000 lux. We observed cell density every 12 hours by counting cells with a hemocytometer and microscope, along with water-quality measurements such as temperature, dissolved oxygen, pH, and salinity.

We adjusted the test doses based on the N/P content of both the Guillard and AGP fertilizers. The results of the preliminary testing that had been conducted showed that the N/P content of each fertilizer was as follows:

Table 1.
Result of N/P Content Analysis

Types of Fertilizer	Content		N/P Ratio
	Nitrogen	Phosporus	
Modified Guillard Fertilizer	395 mg/100 g	96,76 mg/100 g	4 : 1
AGP Fertilizer	3610 mg/100 g	426,55 mg/100 g	9 : 1

The results of the N/P content analysis in Table 1 above were then used as a reference for this study, by increasing the N content in Guillard fertilizer so that the N/P dose was equivalent to the N/P content in AGP fertilizer of 9:1. Furthermore, the effect of different AGP doses on *C. calcitrans* inoculants was tested with 6 treatments, namely: Control (100 ppm modified Guillard fertilizer), and treatments A, B, C, D and E which each consisted of 80, 90, 100, 110 and 120 ppm AGP fertilizer. The AGP dosage range previously used in the hatchery was 100 ppm. Therefore, we tested 80–120 ppm to assess the impact of increasing or decreasing the standard dosage.

2.3. Data Analysis

We analyzed the study data, cell density, and specific growth rate using One-Way Analysis of Variance (ANOVA) at a 5% significance level (95% confidence). If the ANOVA showed that AGP fertilizer at different concentrations had a significant effect, we conducted a Duncan test at a 5% significance level. The Duncan test was used to compare all pairs of treatment means after ANOVA. The specific growth rate and doubling time of *C. calcitrans* can be calculated using the formula used in the research:

$$\mu = \frac{\ln Nt - \ln N0}{Tt - T0} \times 100\%$$

$$dt = \frac{\ln(2)}{\mu}$$

μ = Specific growth rate of microalgae (%/day)
 N_0 = Cell density on day 0 (cells/mL)
 N_t = Cell density on day t (cells/mL)
 T = Time (days) from N_0 to N_t
 dt = Doubling time

III. RESULTS AND DISCUSSION

2.1. Cell Density

The cell density of *C. calcitrans* with AGP fertilizer at different doses showed an average growth value that increased with observation time. The control treatment reached the exponential phase at 72 hours of culture and then entered the stationary phase in subsequent hours. The peak *C. calcitrans* cell density in treatments A, B, C, and D was reached at 108 hours of culture, while in treatment E, the peak density was reached at 120 hours. The results of observations of *C. calcitrans* cell density are presented in Fig. 1. The results of the ANOVA analysis showed that the addition of AGP fertilizer at different doses significantly affected cell density at 0, 12, 24, 36, 48, 60, and 72 hours of culture, but was not significantly different at 84 hours and 96 hours of culture. Furthermore, observations differed significantly again at 108 and 120 hours of culture.

The results of Duncan's test for *C. calcitrans* cell density at 60 hours showed that the highest density was found in *C. calcitrans* cultures in media supplemented with 100 ppm modified Guillard fertilizer, at 164.0×10^4 cells/mL, and the lowest was found in treatment B supplemented with 90 ppm AGP fertilizer, at 69.7×10^4 cells/mL. Based on Duncan's further test, the observation time factor at the beginning and end of the culture formed several homogeneous groups, indicating differences in growth between the observation periods. Treatment E achieved the highest cell density in this study, at 230.0×10^4 cells/mL. Media characteristics in the control treatment initially triggered a very fast specific growth rate but caused nitrogen and phosphorus to be consumed exponentially, so that the cells quickly entered the early stationary phase at 72 hours [10].

Treatments with higher nutrient concentrations tended to have a longer lag phase than the control. This phenomenon is thought to occur because *C. calcitrans* cells need time to adjust their nutrient transport and assimilation mechanisms to nutrient-rich media. At high nutrient concentrations, diatom cells absorb large amounts of nitrogen, phosphorus, and silicate and temporarily store them as food reserves before using them for growth [11]. This accumulation and metabolic adjustment process can slow initial growth, resulting in a longer lag phase.

The test treatment with AGP fertilizer entered the exponential phase at 60 hours of culture. ANOVA tests conducted at 84 hours ($p = 0,256$) and 96 hours ($p = 0,275$) showed no significant differences, indicating that the test treatment with AGP fertilizer had reached its exponential peak and begun to enter the stationary phase, while the control treatment entered the final stationary phase and began to enter the decline phase, resulting in a noticeable difference in cell density. The ANOVA results again showed significant differences at 108 hours ($p = 0,010$) and 120 hours ($p = 0,001$), likely because all AGP fertilizer treatments had entered the exponential phase, while the control treatment was in the final stationary phase and heading toward the decline phase. Nutrient concentration treatments from 25% to 100% increased the maximum density of *C. calcitrans* from 279×10^6 cells/mL to 402×10^6 cells/mL, indicating that greater nutrient availability can support growth in the longer term [12]. Conversely, in the control with lower nutrient levels, cells entered the exponential phase more quickly, but nutrient reserves were depleted more rapidly, resulting in an earlier stationary phase and death.

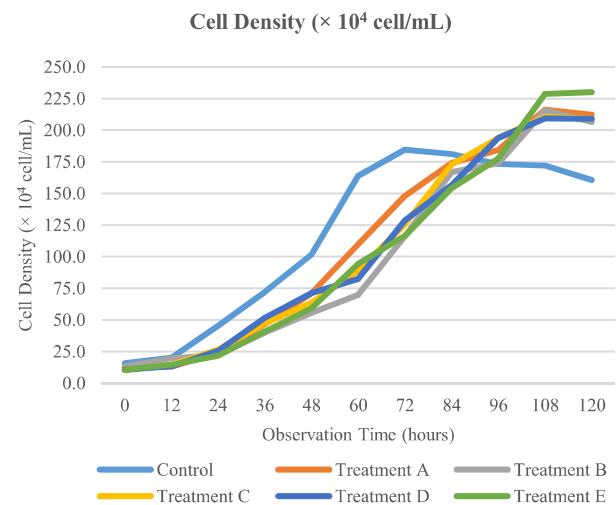


Figure 1. Growth graph of *C. calcitrans* density

2.2. Specific Growth Rate

Based on Table 2, the highest specific growth rate (μ) value was obtained in the control treatment (0.0343 h^{-1}), while all AGP fertilizer addition treatments (80–120 ppm) showed lower μ values, ranging from 0.0255 – 0.0279 h^{-1} . Statistical tests showed that the control differed significantly ($p < 0.05$) from all AGP treatments. The same pattern was observed in doubling time (td), with the control showing the fastest doubling time (20.29 hours), whereas AGP treatments required 24.89–27.27 hours to double the cell number.

The specific growth rate of *C. calcitrans* microalgae across all AGP fertilizer treatments differed significantly

from that of the control treatment. This was due to the peak cell density at 72 hours of the culture period, while treatments A, B, C, and D with AGP fertilizer of 80, 90, 100, and 110 ppm, respectively, reached the highest peak growth phase at 108 hours, while treatment E with 120 ppm was able to reach peak growth at 120 hours. The slow growth rate in the treatment with AGP fertilizer is thought to be due to the availability of nutrients in the media, which is still very abundant, thus creating a longer time for cells to adapt physiologically to balance the process of nutrient absorption and assimilation. The abundance of macronutrients (N, P, K) and micronutrients leads to luxury consumption [11]. *C. calcitrans* cells assimilate and store nutrient reserves in their vacuoles, thereby delaying nutrient starvation stress and extending the exponential growth rate to 120 hours.

The shorter doubling time in the control indicates that cells divide more rapidly under relatively low-nutrient conditions. This indicates that the control medium composition was within an optimal range to support cell division without causing osmotic stress or nutrient imbalance. The specific growth rate and doubling time are inversely proportional, so the higher the μ value, the shorter the time required for cells to double their population. Therefore, the control with the highest μ also showed the lowest t_d . A similar finding was found in the study by [13], which treated with different doses of silicate, phosphorus, and nitrogen, found that increasing the concentration of certain nutrients can increase maximum cell density, but does not always increase the specific growth rate if the cell's nutrient requirements have been met or if a nutrient imbalance occurs.

At 120 hours, the AGP fertilizer treatment had entered the stationary phase, while the control treatment had entered the death phase at 96 hours. This is likely due to depletion of limiting nutrients such as silicate or accumulation of secondary metabolites that inhibit further

cell division in the culture system. Decreased nutrient availability in the culture medium becomes a limiting factor for microalgal growth and cell density [7]. Nitrogen, such as ammonium, is the most efficient form for phytoplankton to assimilate, aiding cell growth. This leads to nutrient uptake from the culture medium, decreasing nutrient concentrations during the culture period. This nutrient limitation ultimately results in phase differences between treatments [14].

Figure 2 shows that *C. calcitrans* reproduces primarily asexually through binary fission (mitosis), producing two identical daughter cells from a single parent cell and providing each daughter cell with the components needed for continued metabolism and growth. After genetic duplication, the cell nucleus divides through mitosis into two identical daughter nuclei. Cytokinesis, the division of the cytoplasm, then separates the parent cell into two daughter cells. Like diatoms, *C. calcitrans* has a silica-rich cell wall (frustule) composed of two valves: the epitheca and the hypotheca [15]. During division, each daughter cell inherits one valve from the parent cell and then synthesizes a new valve within it by depositing silica absorbed from the culture medium. The resulting cells are generally connected by setae (silica spines), forming the chain colonies characteristic of the genus *Chaetoceros*.

Observations of *C. calcitrans* body length in Table 3 showed that the exponential phase in each treatment had the highest observed body length. This is because *C. calcitrans* reproduces by cell division, supported by nutrients in the culture medium. During the exponential phase, cell division is highly active, supported by available nutrients, light, and optimal environmental conditions [16]. However, because new valves form within old valves, average cell size tends to decrease slightly after repeated divisions until, at a minimum size, the diatom can form auxospores to return to the species' maximum cell size.

Table 2.
 Specific Growth Rate Culture

Results	Treatment (hour ⁻¹)					
	Control	Treatment A	Treatment B	Treatment C	Treatment D	Treatment E
Specific Growth Rate (μ)	0,0343±0,0024 ^a	0,0261±0,0003 ^b	0,0255±0,0013 ^b	0,0279±0,0014 ^b	0,0276±0,0020 ^b	0,0256±0,0016 ^b
Doubling Time (t_d)	20,29±1,37 ^a	26,54±0,30 ^b	27,27±1,32 ^b	24,89±1,27 ^b	25,16±1,77 ^b	27,10±1,68 ^b

Note: Different superscripts in each column indicate significant differences ($p < 0.05$)

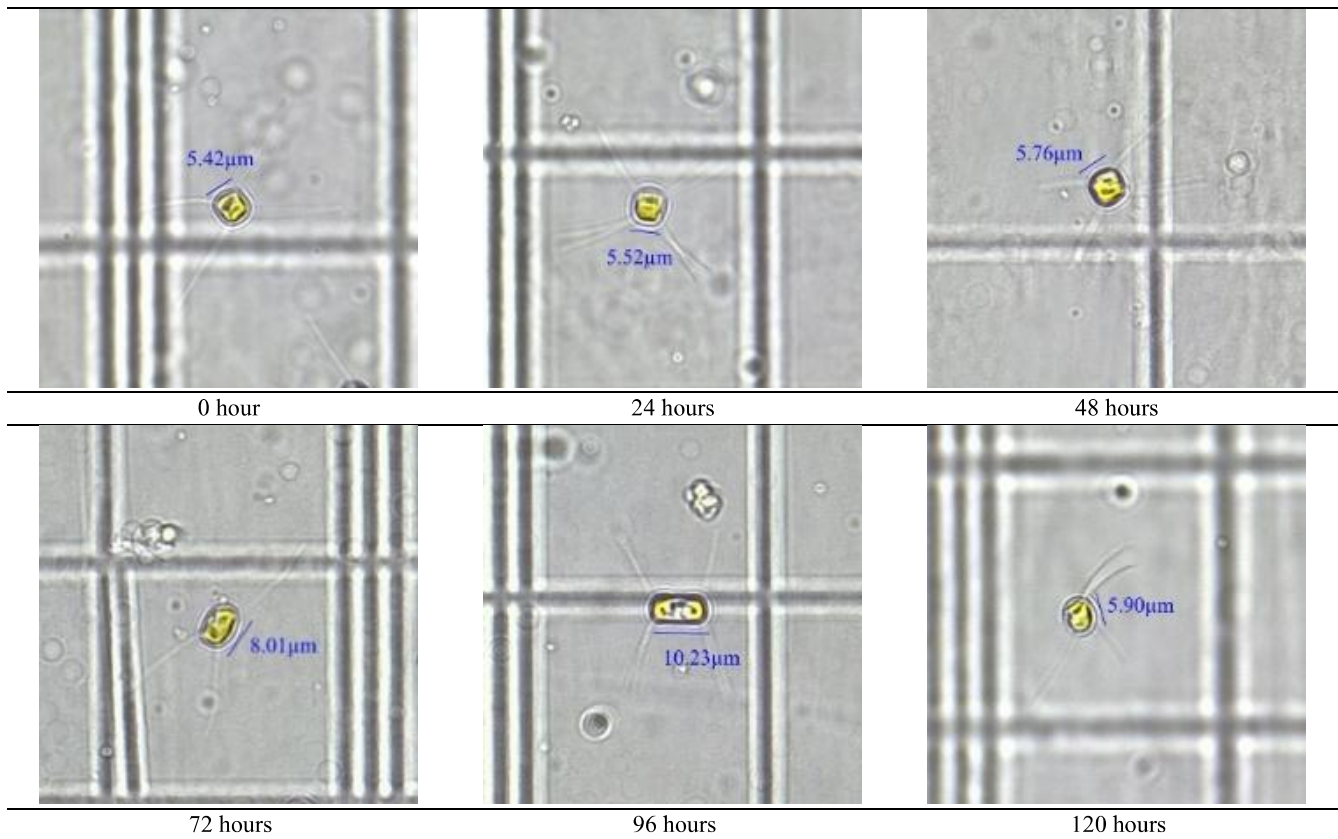


Figure 2. Observation of the size of *C. calcitrans* cells cultured with AGP fertilizer (Magnification of 40×10).

Table 3.

Cell size of *C. calcitrans* during 120th Hours Culture

Treat- ment	Observations Hours (µm)					
	0	24	48	72	96	120
Control	4,9±0,5 ^a	6,7±0,4 ^a	7,8±1,0 ^a	5,9±0,3 ^a	5,7±0,2 ^a	4,7±0,8 ^a
A	4,9±0,7 ^a	5,6±0,8 ^b	6,3±0,8 ^b	7,9±0,3 ^b	6,8±0,7 ^a	4,8±0,1 ^a
B	4,7±0,3 ^a	5,5±0,4 ^b	5,9±0,3 ^b	8,4±0,9 ^b	6,1±0,5 ^a	4,9±0,2 ^a
C	4,7±0,2 ^a	5,4±0,2 ^b	6,1±0,6 ^b	6,5±0,2 ^a	8,6±1,0 ^b	5,8±0,4 ^b
D	4,9±0,6 ^a	5,6±0,2 ^b	6,1±0,2 ^b	6,4±0,3 ^a	9,2±1,0 ^b	5,9±0,6 ^b
E	4,8±0,3 ^a	5,7±0,4 ^b	5,8±0,5 ^b	7,9±0,6 ^b	11,1±0,8 ^c	5,9±0,5 ^b

Note: Different superscripts in each column indicate significant differences (p<0.05)

Water quality measurements over 5 days showed that water temperature ranged from 21.3°C to 25.0°C, dissolved oxygen ranged from 4.22 to 6.87, water salinity in the culture media ranged from 30 to 31, and pH ranged from 7.95 to 8.78. Overall, although the water quality parameter values vary, they remain within the optimum range.

IV. CONCLUSION

AGP fertilizer maximized cell density, entering the exponential growth phase at 48 hours and extending the

stationary phase to 108 hours. However, the control treatment was found to have the best specific growth rate with a μ value of 0,0343 per hour and the highest t_d value of 20,29 among all treatments, while the treatment with AGP fertilizer had the best specific growth rate with a μ value ranging from 0,0255 – 0,0279% per hour and the t_d value ranging from 24,89– 27,27.

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CONFLICT OF INTEREST

The authors have no conflict of interest regarding this publication.

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