

The utilization of whiteleg shrimp (*Litopenaeus vannamei*) aquaculture wastewater in semi-mass *Nannochloropsis* culture

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Abstract. *Nannochloropsis* is a microalga that is used as feed and can be cultured intensively. One of the obstacles in its culture is high-cost production. Innovations that can be applied to overcome this problem is the utilization of wastewater from whiteleg shrimp (*Litopenaeus vannamei*) aquaculture. The purpose of this study was to assess the population growth of *Nannochloropsis* sp. culture by using wastewater of whiteleg shrimp with different densities in a semi-mass culture. The research design used in this study was a completely randomized design with four treatments and three replications, namely treatment A (2.5×10^6 ind/ml), B (5×10^6 ind/ml), C (7.5×10^6 ind/ml), and D (10×10^6 ind/ml). The parameters measured were population density, nitrate, phosphate, pH, temperature, salinity, and light intensity. The results showed that the utilization of wastewater from whiteleg shrimp culture had a significant effect on the population growth of *Nannochloropsis* sp. Treatment A showed the best result with an increase in density by 119% (2.5×10^6 ind/ml to 5.49×10^6 ind/ml) at the adaptation phase, and 1012% (2.5×10^6 ind/ml to 27.8×10^6 ind/ml) at the exponential phase.

1. Introduction

Microalgae is one of the natural feeds that play an important role in shrimp hatchery activities because it can be used as initial feed in the larval or juvenile stage [1]. Various species of microalgae have been studied as a source of nutrients in aquaculture activities, but only a few can be used as natural food [2]. One type of microalgae that is useful as initial feed and can be cultured intensively is *Nannochloropsis* sp. *Nannochloropsis* sp. is one of the microalgae that contains 34-41% protein, 7-13% carbohydrates, and 7-9% lipids ([3]. Also, *Nannochloropsis* sp. does not cause toxins and damage to larval rearing tanks, relatively fast growth, has contained antibiotics and can adsorb toxic substances such as Lead (Pb) and Mercury (Hg) [4].

One of the obstacles in *Nannochloropsis* sp. culture is the high cost of production. This is due to efforts to fulfil nutrients in the culture media of *Nannochloropsis* sp. still use expensive Conway or Walne fertilizers [5]. Complete nutrient composition and proper nutrient concentration will greatly determine biomass production and nutrient content of microalgae [6]. Therefore, alternative culture media is needed as a source of macronutrients and micronutrients that can meet the nutritional needs of *Nannochloropsis* sp. during culture.

One alternative that can be used is shrimp wastewater aquaculture. Shrimp wastewater aquaculture is liquid waste generated from the process of changing water from shrimp aquaculture ponds [7].

Shrimp wastewater aquaculture contains inorganic materials such as N and P which can be utilized by *Nannochloropsis* sp. as a nutrient. The results of previous studies [8] indicated that it was contained with 12,388 mg/l total organic C; 2,199 mg/l total N; and 0.660 mg/l total P, where the levels of nitrate and phosphate contained are influenced by the feed given during shrimp farming activities. In addition, the use of wastewater from shrimp culture as an alternative medium in the culture of *Nannochloropsis* sp. can be a solution in utilizing shrimp pond wastewater to improve the environment and ecosystem around the pond. It is expected that the utilization of whiteleg shrimp (*Litopenaeus vannamei*) wastewater aquaculture can produce the highest cell density and the fastest specific growth rate in semi-mass microalgae culture activities of *Nannochloropsis* sp. The purpose of this study was to assess the population growth of *Nannochloropsis* sp. culture by using wastewater of whiteleg shrimp with different densities in a semi-mass culture

2. Material and methods

2.1. Sample preparation

Nannochloropsis sp. was obtained from the Marine Aquaculture and Fisheries Center of Lampung (BBPBL). Shrimp wastewater came from the whiteleg shrimp (*Litopenaeus vannamei*) farm in Kalianda-South Lampung. The research design used in this study was a completely randomized design with three replications and four treatments with different density, namely treatment A (2.5×10^6 ind/ml), B (5×10^6 ind/ml), C (7.5×10^6 ind/ml), and D (10×10^6 ind/ml).

The procedure in this study is divided into several stages, tool sterilization, preparation of culture containers, preparation of *Nannochloropsis* sp. inoculum, calculation of initial density, distribution of *Nannochloropsis* sp. inoculum, calculation of population density, measurement of water parameters quality (temperature, pH, salinity, nitrate, and phosphate) following the previous studies [9]. The *Nannochloropsis* sp. inoculum was put into each culture container at a density of 10^6 ind/ml.

2.2 Research parameter measurement

The parameters measured were population density, nitrate, phosphate, pH, temperature, salinity, and light intensity. The calculation formula for population density was adapted from the following method [10]:

$$\text{Density} = \frac{\text{N in 4 block}}{\text{block number (4)}} \times 10^4 \text{ cell/ml}$$

In this study, the nitrate measurement was performed following SNI 06-2480-1991 method, while the phosphate used SNI 06-6989.31-2005 method [11], [12]. Other water quality parameters used methods from previous studies [13], [14].

3. Result and discussion

3.1. *Nannochloropsis* sp. Density

Data on *Nannochloropsis* sp. density on semi-mass cultured using 100% wastewater media from whiteleg shrimp culture with different inoculum densities could be seen in Figure 1. The results indicated that whiteleg shrimp wastewater could increase the growth of *Nannochloropsis* sp. This was due to the availability of sufficient nutrients in the culture media, so the population density of *Nannochloropsis* sp. grew faster. The results of previous research [8] noted that the wastewater of whiteleg shrimp culture contained 12.388 mg/l of total organic C; 2.199 mg/l total N; and 0.660 mg/l total P. Nutrients were required by *Nannochloropsis* sp. to reproduce and increase its population density [15].

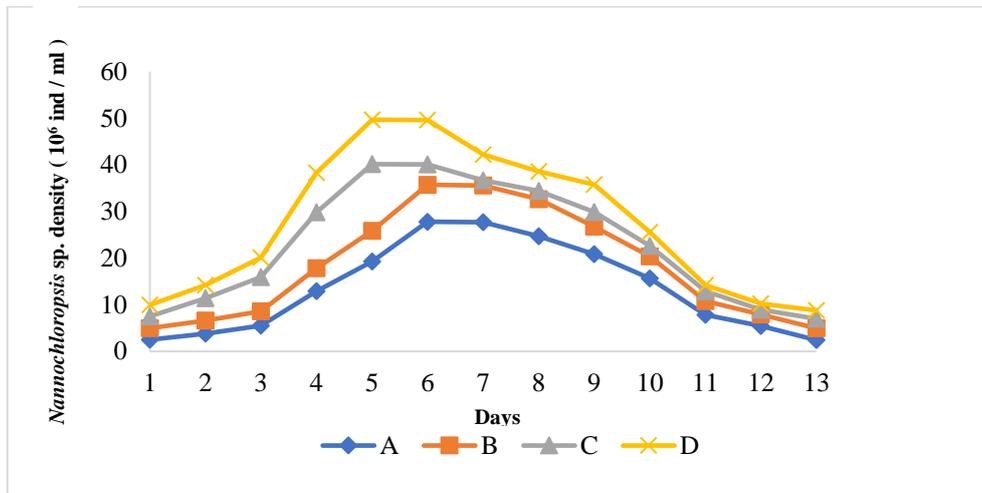


Figure 1. Population density of *Nannochloropsis* sp. in culture with different densities inoculation. Note the density inoculation A. 2.5×10^6 ind/ml, B. 5×10^6 ind/ml, C. 7.5×10^6 ind/ml, and D. 10×10^6 ind/ml.

Table 1. Percentage of *Nannochloropsis* sp. population.

Treatment	Adaptation Phase (%)	Exponential Phase (%)
A (2.5×10^6 ind/ml)	119	1012
B (5.0×10^6 ind/ml)	105	614
C (7.5×10^6 ind/ml)	112	435
D (10×10^6 ind/ml)	100	297

The results indicated that the increase in treatment A (2.5×10^6 ind/ml) resulted in optimal biomass and density compared to others (Table 1.). This was due to the low inoculum density, thus the utilization of macronutrients and micronutrients by *Nannochloropsis* sp. is more optimal in the culture media, where the microalgae did not need to compete for nutrients [16], [17].

3.2. Water Parameter Measurement

A significant reduction in nitrate levels occurred in treatment D (10×10^6 ind/ml), with a reduced rate of 6.8 mg/l during the study period from the start of culture (day 0) to the end of culture (day 13) with a percentage reduction of 79.0% (Table 2.). While the lowest decrease in nitrate levels occurred in treatment A (2.5×10^6 ind/ml), with a decrease in the level of 4.5 mg/l during the study period from the beginning of culture (day 0) to the end of culture (day 13) with a decreasing percentage of 54.2%.

Table 2. Nitrate and phosphate levels in media of *Nannochloropsis* sp.

Treatment	NO ₃ (mg/l)		Total Decrease (mg/l)	Decrease Percentage (%)	PO ₄ (mg/l)		Total Decrease (mg/l)	Decrease Percentage (%)	N:P	
	T ₀	T ₁₃			T ₀	T ₁₃			T ₀	T ₁₃
A	8.3	3.8	4.5	54.2	9.2	4.0	5.2	56.5	1 : 1.1	1 : 1
B	8.4	3.0	5.3	63.0	9.4	3.2	6.2	65.9	1 : 1.1	1 : 1
C	8.7	2.4	6.3	72.4	9.5	2.4	7.1	74.7	1 : 1.1	1 : 1
D	8.6	1.8	6.8	79.0	9.6	2.0	7.5	78.1	1 : 1.1	1 : 1,1

The reduced levels of nitrate were caused by *Nannochloropsis* sp. utilize nitrate as a nutrient to support its growth. Nitrate was the main nutrient for microalgae growth [18]. Previous studies had shown that nitrates played a role in the process of amino acids, fats, and vegetative cells formation [19].

A significant decrease in phosphate levels occurred in treatment D (10×10^6 ind/ml). The decrease occurred as much as 7.5 mg/l with a percentage decrease of 78.1%. The lowest decrease in phosphate levels occurred in treatment A (2.5×10^6 ind/ml). In treatment A, a decrease in phosphate levels occurred as much as 5.2 mg/l with a decrease in the percentage of 56.5%. A decrease in phosphate levels indicated that absorption of orthophosphate nutrients by *Nannochloropsis* sp. was to be able to meet nutritional needs and support Microalgae used inorganic phosphorus in the form of orthophosphate for the process of forming proteins, carbohydrates, cell structures, and cell membrane stabilizers [19].

Nannochloropsis sp. had a close relationship with the availability of N and P nutrients in culture media. In addition to the N and P concentrations, the N/P ratio affected the population density of *Nannochloropsis* sp. The N/P ratio was used to determine which nutrients limited the growth of *Nannochloropsis* sp. The Redfield criterion for the N/P ratio, namely, $N/P < 16$ indicated that N was a limiting factor, if $N/P > 16$ then P became the limiting factor, and if N/P was 14-16 then N or P collectively became the limiting factor [21]. Based on the overall N/P ratio in each treatment (Table 2), the nutrient which was the limiting factor in this study was Nitrate. This showed that the growth of *Nannochloropsis* sp. would be hampered by limited nitrate levels in the culture media.

Table 3. Temperature, pH, salinity and light intensity measurement from *Nannochloropsis* sp. media.

Parameter	Treatment								Optimum
	A		B		C		D		
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	
Temperature (°C)	26	29	26	29	26	29	26	30	25-30 [21]
pH	6.8	8.3	6.7	8.4	6.7	8.6	6.8	8.8	6-9.5 [21]
Salinity (ppt)	36	39	36	39	36	39	36	39	30-36 [21]
Light intensity (lux)	3600	4230	3600	4256	3600	4245	3600	4275	1000-10000 [22]

Measurement of water quality showed that temperature, pH, and light intensity still conform with the optimum conditions for *Nannochloropsis* sp. growth, except for salinity (Table 3.). *Nannochloropsis* sp. growth is greatly influenced by environmental factors, such as salinity. The optimum salinity ranged for the growth of *Nannochloropsis* sp. which was 30-36 ppt. Changes in salinity and CO₂ at the exponential phase could increase density and change lipid content. The increase in salinity levels was caused by water evaporation which reduced the volume of water so that the concentration of dissolved salts in it increases. The combination of increasing salinity and decreasing nitrogen was able to produce high lipids of 31.45% [23]. Increased salinity with normal nitrogen resulted in increased total protein content [24].

4. Conclusion

The results showed that the utilization of wastewater from whiteleg shrimp culture had a significant effect on the population growth of *Nannochloropsis* sp. Treatment A showed the best result with an increase in density by 119% (2.5×10^6 ind ml to 5.49×10^6 ind/ml) at the adaptation phase, and 1012% (2.5×10^6 ind/ml to 27.8×10^6 ind/ml) at the exponential phase.

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