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Impact of Different Nutrient Enrichment Concentrations on the Growth of Microalga *Nannochloropsis* sp. (Monodopsidaceae) Culture

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ABSTRACT

Microalgae consist of unicellular algal species that can produce and accumulate a wide variety of biomolecules. In order to maintain a high cell density in a continuous phototrophic culture of algae, the nutrient can serve as the most important factor in enhancing cell density. In this study, the effect of different concentrations of nutrients on the cell density of microalga *Nannochloropsis* sp. cultured in the mega plastic box (with 50 L capacity) was investigated. Four groups of treatment with four replicates were tested: group A (including 5 g L^{-1} of ferric chloride, ammonium phosphate, and Urea), group B (including 10 g L^{-1} of ferric chloride, ammonium phosphate, and urea), group C (including 15 g L^{-1} of ferric chloride, ammonium phosphate, and urea), and group D (including 15 g L^{-1} of ferric chloride, ammonium phosphate, and urea with 40 g L^{-1} of cow manure). Results revealed that group C and group D achieved maximum density on day three as 86.39×10^6 cell mL^{-1} and 85.59×10^6 cell mL^{-1}, respectively, which were significantly ($p\leq0.05$) higher than the cell density of groups A (58.01×10^6 cell mL^{-1}) and group B (70.67×10^6 cell mL^{-1}). Additionally, the increasing specific growth rate (SGR) of *Nannochloropsis* sp. cultured was obtained in group D at 0.308 day^{-1} after the culture period. From the result of the study, it is concluded that the concentrations of 15 g L^{-1} ferric chloride, ammonium phosphate, and urea (group C) and 15 g L^{-1} ferric chloride, ammonium phosphate, and urea combined with 40 g L^{-1} cow manure (group D) are capable of increasing cell density growth of microalga *Nannochloropsis* sp. cultured in a mega plastic box.

INTRODUCTION

Microalgae can be used in many biotechnological applications, including health foods, feeds, colorants, bioenergy, and pharmaceuticals (Pulz & Gross, 2004; Mortensen, 2006; Chu, 2012; Dixit & Suseela, 2013). Microalgal compounds are particularly valuable for finding new medicines that treat viral infections, cancers, and bacteria and fungi showing resistance to antibiotic treatments (Skulberg, 2000; Huleihel et al., 2001). In addition, various microalgae contain DHA (docosahexaenoic acid), which contributes to heart health in adults and brain development in babies (Pulz & Gross, 2004). In addition to their role in photosynthesis, microalgal pigments have a wide range of biological properties, including antioxidants (Hejazi et al., 2004; Chidambara-Murthy et al., 2005), anti-obesity, anti-angiogenic, anti-cancer, and neuroprotective properties (Ciccone et al., 2013), as well as an anti-inflammatory (García-González & Ochoa, 1999; Guzman et al., 2001). Growing concern about human nutrition has led to controversy regarding microalgae in poultry and fish (Souza et al., 2021). Furthermore, researchers examined how microalgae can be cultivated rapidly and produced in large quantities (Duong et al., 2012; Leu & Boussiba, 2014; Mohan et al., 2015). Various culturing systems have been used to cultivate microalgae at large scales under controlled conditions, including polyethylene sleeves, outdoor ponds, and tubular bioreactors (Lebeau & Robert, 2003; Benner et al., 2022). Moreover, microalgal growth is influenced by environmental parameters such as temperature, light intensity, and photoperiod in culture systems (George et al., 2014). There are a number of factors that affect microalgae growth and biochemical composition, including nutrients (Juneja et al., 2013; Barkia et al., 2019). The manure of terrestrial animals can have significant effects on plants, including macroalgae (Jjemba, 2002; Bogaard et al., 2007; Giwa, 2017). However, the studies related to the production of microalgae with manure and other inorganic nutrients are very limited. Thus, this study examines the effect of different concentrations of nutrients along with cow manure on the growth of *Nannochloropsis* sp. culture.

MATERIAL AND METHODS

Study Area

The experiment was conducted at the Multi-species Hatchery, situated in the College of Fisheries, Mindanao State University-Tawi-Tawi College of Technology and Oceanography (MSU-TCTO), Sanga-Sanga, Bongao, Tawi-Tawi, Philippines (05°02'3.8" N 199°44'5.0" E) on March 23-27, 2022.

Culture Condition

Mega plastic boxes with 50 L volume were used in *Nannochloropsis* sp. experiment. Chlorinated seawater was used in this study and filtered through a filter bag before being transported to 16 mega boxes containing 45 liters each. After the 16 mega boxes were filled with chlorinated seawater, starter green microalgae of *Nannochloropsis* sp. with 5 L volume inoculated into the culture container. Mega plastic boxes were then mixed with four different nutrient concentrations randomly (Table 1) with four replicates for each treatment. The mega plastic boxes were covered with transparent acetate film to avoid contamination, and fluorescent lamps (Ecolum LED) were used as artificial lighting for the cultures (Figure 1).

Analysis

A daily sample of *Nannochloropsis* sp. culture was taken for cell counting and analysis. The number of cells was counted by using a hemocytometer, and contamination was investigated under a microscope. Specific growth rates (μ) were calculated by following the formula (Durmaz & Erbil, 2017);

$$\mu = \frac{\ln(N_2)-\ln(N_1)}{t_2-t_1} \quad (1)$$

where: N_2 is the biomass cell number at the time (t_2). N_1 is the beginning biomass cell number at the time (t_1).

IBM SPSS software version 20 was used to analyze the significance of differences between the growth using one-way analysis of variance (ANOVA). Post hoc (Duncan) was used to rank the means.

Table 1. Different concentrations of nutrients of ferric chloride, ammonium phosphate, urea, and cow manure that used in four different experimental treatment groups

Treatment	Ferric Chloride (g L^{-1})	Ammonium Phosphate (g L^{-1})	Urea (g L^{-1})	Cow Manure (g L^{-1})
Group A	5	5	5	0
Group B	10	10	10	0
Group C	15	15	15	0
Group D	15	15	15	40

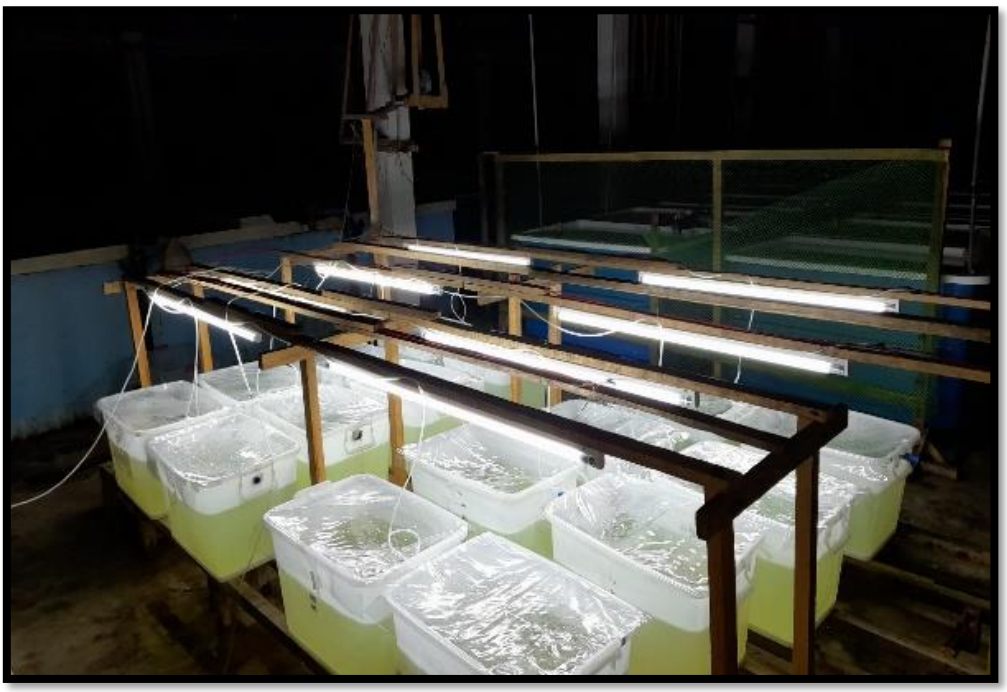

Figure 1. Experimental set-up

RESULTS

The initial cell density of *Nannochloropsis* sp. was arranged as 3.20×10^6 cell mL^{-1} (Figure 2). The microalga *Nannochloropsis* sp. was enriched with different concentrations of nutrients. All of the experimental groups reached the maximum cell density on the 3rd day of culture. The maximum cell density of groups C and D on 3rd day of the culture period was 86.39×10^6 cell mL^{-1} and 85.59×10^6 cell mL^{-1}, respectively, which was significantly ($p\leq0.05$) higher than the cell density of groups A and B at 58.01×10^6 cell mL^{-1} and 70.67×10^6 cell mL^{-1}, respectively. The lag phase was observed on the first day for all the group treatments. After the first day, the cell density of *Nannochloropsis* sp. in group A increased rapidly from 29.55×10^6 cell mL^{-1}, reaching 50.01×10^6 cell mL^{-1}, while in group B, the cell density increased from 27.59×10^6 cell mL^{-1} to 70.67×10^6 cell mL^{-1} by day 3 without any apparent lag phase. Moreover, the cell density in group C was increased from 27.97×10^6 cell mL^{-1} to 86.39×10^6 cell mL^{-1}, while in group D it was increased from 31.05×10^6 cell mL^{-1} to 85.59×10^6 cell mL^{-1} at the 3rd day. Furthermore, the specific growth rate (SGR) of *Nannochloropsis* sp. culture is shown in Figure 3. The SGR of *Nannochloropsis* sp. culture in group D (0.308 ± 0.003 day^{-1}) was increased than the SGR in group A (0.170 ± 0.011 day^{-1}), B (0.173 ± 0.011 day^{-1}), and C (0.188 ± 0.019 day^{-1}) at the end of culture period.

DISCUSSION

The primary aim of producing phototrophic organisms is to maintain a high cell density in a continuous culture. In the present study, green microalga *Nannochloropsis* sp. was investigated in

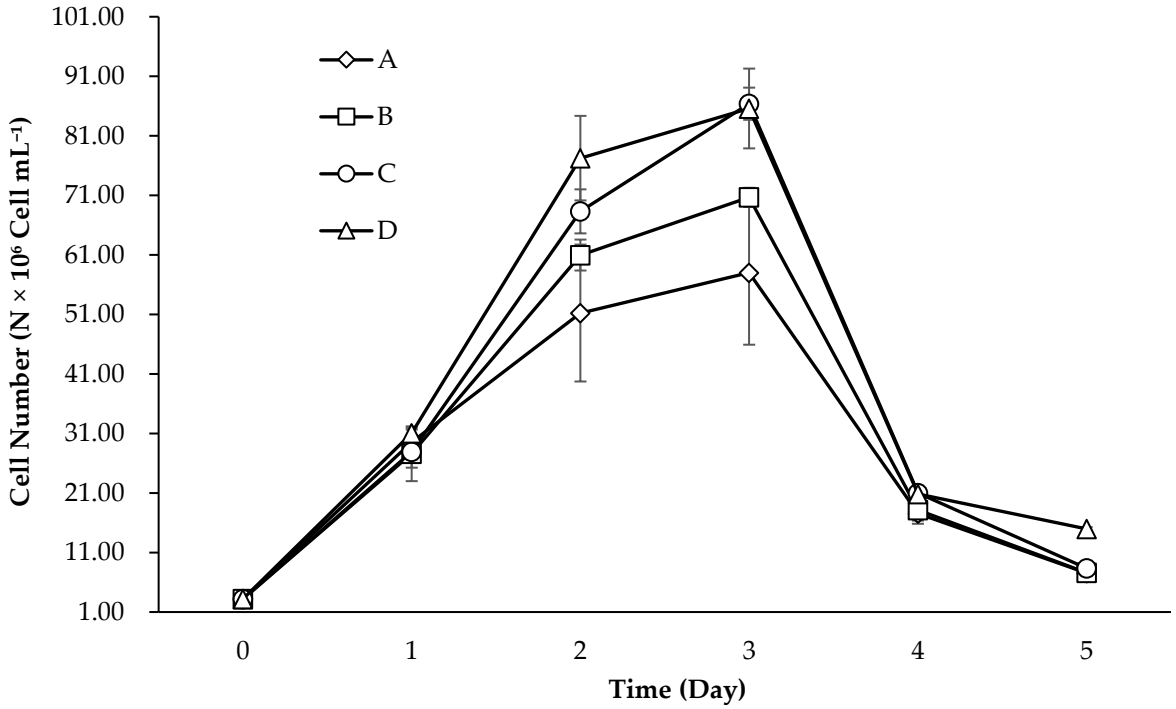

Figure 2. Cell densities of different concentrations of nutrient enrichment. A = (5 g L^{-1} of ferric chloride, ammonium phosphate, and Urea); B = (10 g L^{-1} of ferric chloride, ammonium phosphate, and Urea); C = (15 g L^{-1} of ferric chloride, ammonium phosphate, and Urea); D (15 g L^{-1} of ferric chloride, ammonium phosphate, and Urea with 40 g L^{-1} of cow manure), N=16.

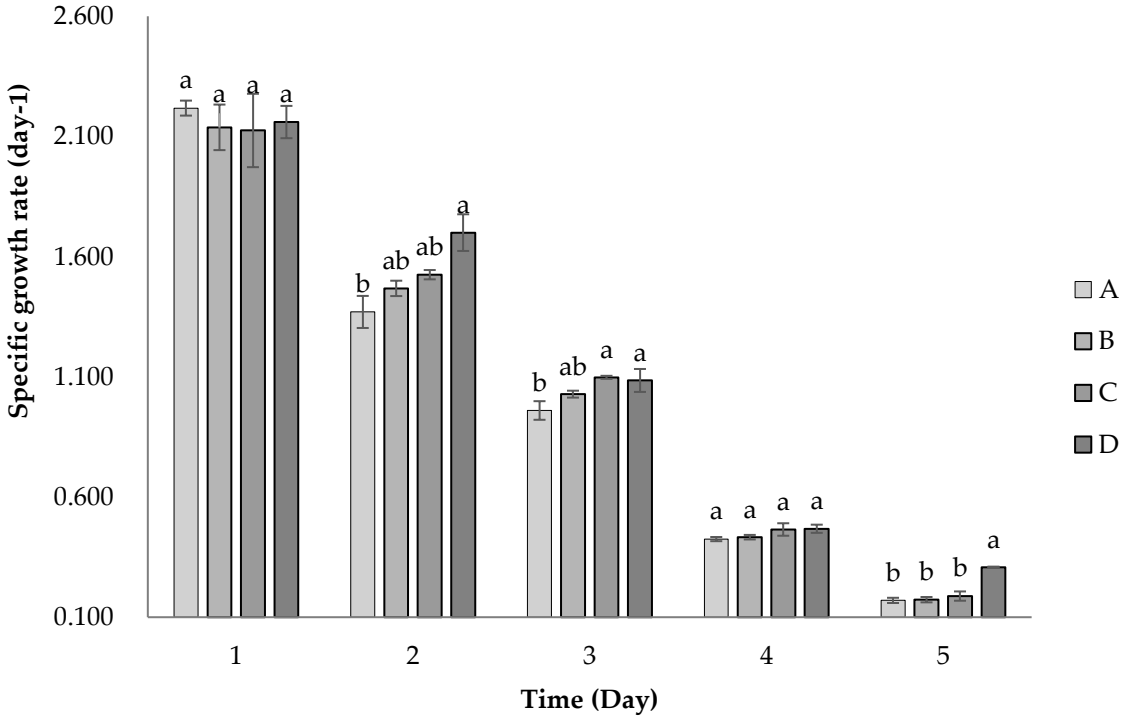

Figure 3. Specific growth rate of different concentrations of nutrient enrichment. A = (5 g L^{-1} of ferric chloride, ammonium phosphate, and Urea); B = (10 g L^{-1} of ferric chloride, ammonium phosphate, and Urea); C = (15 g L^{-1} of ferric chloride, ammonium phosphate, and Urea); D (15 g L^{-1} of ferric chloride, ammonium phosphate, and Urea with 40 g L^{-1} of cow manure). Bar with the same letters is not significantly different ($p \leq 0.05$), Error bars in SEM (standard error mean) N=16.

response to different concentrations of nutrients. Utilizing 15 g L^{-1} ferric chloride, ammonium phosphate, and urea (group C) and 15 g L^{-1} ferric chloride, ammonium phosphate, and urea combined with 40 g L^{-1} cow manure (group D), the maximum cell density reached 86.39×10^6 cell mL^{-1} and 85.59×10^6 cell mL^{-1}, respectively, culture in a mega plastic box (50 L capacity). While increasing SGR of *Nannochloropsis* sp. culture was obtained in group D at 0.308 day^{-1} after the culture period. Another study conducted bag cultivation (50 L) for microalga *Nannochloropsis oculata* cultured in F/2 medium enriched with different concentrations of nitrogen sources such as sodium nitrate ($NaNO_2$) and ammonium chloride (NH_4Cl) where the highest number of cells was given as 52×10^6 cell mL^{-1} and 49×10^6 cell mL^{-1}, respectively, at 881 µmol L^{-1} (Durmaz, 2007). As a result of microalga *N. oculata* culture in a 60 L photobioreactor, harvest cell density in F/2 medium supplemented with 1.76 µmol L^{-1} of nitrogen was 52.8×10^6 cell mL^{-1} (Huang et al., 2013). Low & Toledo (2015) examined that the cell density from 80 L bag culture of microalga *N. oculata* has been harvested at an approximate concentration of 4.55×10^6 cell mL^{-1}. Additionally, fiberglass reinforced plastic panel photobioreactor for microalga *N. oculata* cultured in F/2 medium obtained the highest density of 245×10^6 cell mL^{-1} (Durmaz & Erbil, 2020). It has also been reported that *N. oculata* cultured in 6 L flasks enriched with F/2 medium and supplemented with 100 mg L^{-1} and 500 mg L^{-1} concentrations of myo-inositol achieved maximum densities of 101.00×10^6 cell mL^{-1} and 91.33×10^6 cell mL^{-1}, respectively (Erbil & Durmaz, 2020). Other researchers stated that microalga *Porphyridium cruentum* cultured in flat bottom flasks (1 L) enriched with F/2 medium and combined with 0.585×10^{-5} M ferric chloride achieved a maximum density of 5.39×10^6 cell mL^{-1} (Erbil et al., 2022). Furthermore, it has been demonstrated that high-nutrient manures, such as pig and poultry manures and bi-products of anaerobic digestion, may be viable sources of nutrients for microalgae cultivation (Fenton, 2012; Lu & Xiao, 2022). Our study demonstrated that a combination of cow manure and other inorganic nutrients, such as ferric chloride and ammonium phosphate, significantly increased the microalgae cell density.

CONCLUSION

Nannochloropsis sp. is an important species in aquaculture hatcheries because of its phototrophic nature, enabling a continuous culture of high cell-density organisms. As a result of the present study, the maximum cell density growth of *Nannochloropsis* sp. was significantly increased in both the 15 g L^{-1} ferric chloride, ammonium phosphate, and urea (group C) and the 15 g L^{-1} ferric chloride, ammonium phosphate, and urea combined with 40 g L^{-1} cow manure (group D) at three days of the culture period.

Compliance with Ethical Standards

Authors' Contributions

NBS: Manuscript design, laboratory experiment, and draft checking.

MDH, CTN, RJFR, HAI, JSM, & JHE: Manuscript design and draft checking.

JHS: Statistical analyses, writing, draft checking, reading, and editing.

All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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