JIPK. Volume 17 No 3 October 2025
Sinta 1 (Decree No: 158/E/KPT/2021)
e-ISSN:2528-0759; p-ISSN:2085-5842
Available online at https://e-journal.unair.ac.id/JIPK

JIPK
(JURNAL ILMIAH PERIKANAN DAN KELAUTAN)
Scientific Journal of Fisheries and Marine

Research Article

Evaluation of AMPEP as a Natural Biostimulant for Enhancing Biomass and Pigment Yield in *Chlorella sorokiniana*

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OPEN ACCESS

ARTICLE INFO

Received: July 03, 2025
Accepted: August 26, 2025
Published: Sept 22, 2025
Available online: Sep 27, 2025

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Keywords:
AMPEP
Cell density
Cell size
Pigment
Chlorella sorokiniana

Abstract

Chlorella sorokiniana is a promising microalga valued for its production of pigments, lipids, and proteins with potential applications in biofuels, nutraceuticals, and pharmaceuticals. However, enhancing its growth and productivity remains a key challenge. Acadian Marine Plant Extract Powder (AMPEP), derived from the brown seaweed *Ascophyllum nodosum*, is known for its growth-promoting and stress-resistance properties in plants, but its effects on microalgae are not well understood. This study aimed to evaluate the effects of different concentrations of AMPEP (50, 100, 150, and 200 mg L^{-1}) on the growth, biomass, and pigment accumulation of *C. sorokiniana.* The experiment was conducted using a completely randomized design with five treatments (including a control) and three replicates per treatment. The results showed that 100 mg L^{-1} AMPEP produced the highest cell density, with a 2.50-fold increase compared to the control, and the highest specific growth rate of 0.17 ± 0.03 day^{-1}. The largest cell size (19.51 ± 0.77 µm) was recorded at 200 mg L^{-1}, while biomass production peaked at 6.41 ± 0.49 g L^{-1} with 50 mg L^{-1}. Maximum chlorophyll a and total carotenoid content were observed at 150 mg L^{-1}. Overall the 100 mg L^{-1} AMPEP is the most balanced and optimal concentration overall for growth enhancement of *C. sorokiniana,* while other concentrations may be selected based on specific objectives like pigment or biomass production. These findings suggest that AMPEP, particularly at moderate concentrations, can significantly enhance the growth, biomass yield, and pigment content of *C. sorokiniana.* Further research is recommended to investigate the underlying mechanisms of AMPEP's biostimulant effects and its potential application in large-scale algal cultivation systems.

Cite this as: Jalilul, J. N. M., Jeva, M. A., Sarri, J. H., Robles, R. J. F., & Jamil, W. M. (2025). Evaluation of AMPEP as a Natural Biostimulant for Enhancing Biomass and Pigment Yield in *Chlorella sorokiniana*. *Jurnal Ilmiah Perikanan dan Kelautan*, 17(3):724-735.https://doi.org/10.20473/jipk.v17i3.75369

1. Introduction

Microalgae are photosynthetic organisms with remarkable adaptability, capable of thriving in diverse aquatic environments ranging from oceans to wastewater treatment systems (Khan *et al*., 2018). These unicellular organisms can withstand variations in temperature, salinity, pH, and light intensity, making them versatile candidates for a range of ecological and biotechnological applications (Barsanti and Gualtieri, 2022; Manning and Gol, 2021). Biostimulants play a crucial role in optimizing microalgae cultivation by enhancing growth, biomass production, and stress tolerance through natural, eco-friendly mechanisms (Miranda *et al.,* 2024; Brito-Lopez *et al.*, 2025). Derived from sources like seaweed extracts, humic substances, and microbial derivatives, biostimulants promote physiological processes such as nutrient uptake, enzyme activation, and photosynthetic efficiency (El Boukhari *et al.,* 2020; Ali *et al.,* 2021). Optimizing microalgae growth and productivity is essential for large-scale applications like biofuels and nutraceuticals. However, maintaining ideal conditions (light, nutrients, CO_2, etc.) in controlled systems at scale is challenging and costly. While lab setups offer precision, scaling up often leads to inconsistent results, contamination, and high operational demands, making commercial production difficult.

In recent years, microalgae have gained increasing attention for their capacity to produce high-value compounds such as pigments, lipids, and proteins, with potential uses in biofuels, pharmaceuticals, nutraceuticals, and functional foods (Chisti, 2007; Gaurav *et al.,* 2024). In the field of aquaculture, they are essential in maintaining water quality, performing bioremediation, and serving as a primary live feed for larval stages of aquatic organisms (Shuba and Kifle, 2018). Moreover, microalgal pigments particularly carotenoids such as β-carotene have shown promising antioxidant, neuroprotective, and hepatoprotective properties, which are being explored for health-related applications (Saide *et al*., 2021; Anusree *et al.,* 2023).

Among the widely studied microalgal genera, *Chlorella* specifically *Chlorella sorokiniana* has received significant attention due to its fast growth, rich pigment content, and commercial potential (Montoya-Vallejo *et al.,* 2023). This species is particularly noted for producing chlorophyll a and various carotenoids, which contribute to both photosynthetic efficiency and health-related benefits, such as antioxidant activity and potential protective effects against chronic diseases (Morais *et al.,* 2024; Liu *et al.,* 2021). Furthermore, chlorophyll pigments are being investigated as natural food colorants, offering a sustainable alternative to synthetic dyes in the food industry (De Clerck *et al.,* 2013; Sarri *et al.,* 2024c). To enhance pigment yield and biomass productivity in *C. sorokiniana*, studies have focused on manipulating culture conditions including nutrient composition, light regimes, and CO_2 availability, as well as modifying media such as BG-11 to improve growth performance (Dahiya *et al.,* 2021). This statement establishes the broader context of strategies employed to enhance pigment yield and biomass in *C. sorokiniana*, including optimizing nutrient composition and modifying culture media. This research directly contributes to this area by exploring the use of Acadian Marine Plant Extract Powder (AMPEP) as a biostimulant supplement to the BG-11 medium, aiming to improve growth and pigment production, thereby supporting the overall goal of optimizing culture conditions for microalgal productivity.

Despite advances in optimizing physical and chemical cultivation parameters, the use of natural biostimulants such as Acadian Marine Plant Extract Powder (AMPEP) has rarely been explored in microalgal systems. AMPEP, derived from the brown seaweed *Ascophyllum nodosum*, is known for enhancing growth and stress resistance in terrestrial plants and marine organisms (Rouphael and Colla, 2020; Khan *et al*., 2018). However, its effects on microalgae, particularly on *Chlorella sorokiniana*, are presently unclear. Few data are available regarding the optimal concentrations or application strategies of AMPEP in algal cultures, and its physiological impacts on growth dynamics and pigment accumulation remain not well understood.

This study aimed to evaluate the effect of AMPEP at different concentrations (50, 100, 150, and 200 mg L^{-1}) on the growth performance, biomass yield, and pigment production of *C. sorokiniana*. The outcome of this research provides new insight into the potential of AMPEP as a natural biostimulant for enhancing microalgal productivity and offers practical implications for its application in biotechnological and aquaculture industries. The scope is limited to *Chlorella sorokiniana* cultured under controlled laboratory conditions using. Furthermore, the novelty of applying AMPEP in microalgal cultivation means that available literature for direct comparison is scarce, making the interpretation of results largely exploratory.

2. Materials and Methods

2.1 Materials

2.1.1 The equipment

The experimental setup included 500 mL glass bottles for culturing, a YX24LOJ portable autoclave for sterilization, and syringe filters (0.2 µm) to ensure sterility. Fluorescent lamps provided continuous lighting,

and an air motor was used for aeration. A room air conditioner maintained the temperature at 20 ± 1°C. Cell analysis involved a light microscope, Neubauer hemocytometer, and ImageJ software. Biomass was measured using a drying oven, and pigment analysis was performed with a centrifuge, vortex mixer, and spectrophotometer. Data analysis was conducted using IBM SPSS version 20.

2.1.2 The materials

The study used *Chlorella sorokiniana* as the test microalga, cultured in BG-11 nutrient medium composed of essential macronutrients ($NaNO_3$, K_2HPO_4, $MgSO_4 \cdot 7H_2O$, $CaCl_2 \cdot 2H_2O$, etc.) and trace elements (H_3BO_3, $MnCl_2 \cdot 4H_2O$, $ZnSO_4 \cdot 7H_2O$, etc.). Acadian Marine Plant Extract Powder (AMPEP), derived from *Ascophyllum nodosum* (Table 1) was applied at concentrations of 0, 50, 100, 150, and 200 mg L^{-1}. These concentrations were chosen based on a preliminary study conducted to determine a suitable range for evaluating the effects of AMPEP on *C. sorokiniana*. Methanol was used for pigment extraction, and distilled water was used for preparing media and dilutions.

Table 1. Composition of Acadian marine plant extract powder (AMPEP) 0.7 – 0.09 – 14.1 from *Ascophyllum nodosum* (The composition was obtained from the Acadian sea plants, product of Canada) (Sarri *et al.,* 2024b)

Physical analysis	
Appearance	Brownish-black crystals
Odor	Marine odor
Solubility in water	100%
Typical analysis	
Minerals (Ash)	45-50%
Maximum moisture	6.5%
Minimum alginic acid	10%
Minimum Mannitol	4%
Minimum Amino acids	4%
Nitrogen (N) as organic	0.7%
Phosphorus (P) as water-soluble	0.09%
Total potassium (K)	14.1%

2.1.3 Ethical approval

This study does not require ethical approval because it does not use experimental animals.

2.2 Methods

The experiment was carried out for 24 days at the Marine Integrated Laboratory, Mindanao State University Tawi-Tawi College of Technology and Oceanography, Philippines. *Chlorella sorokiniana* was cultured in sterile 500 mL glass bottles containing BG-11 medium, with AMPEP added at concentrations of 50, 100, 150, and 200 mg L^{-1}. The control group sample does not contain any source of AMPEP. The cultures were maintained at 20 ± 1°C with constant aeration, and light intensity was subjected to continuous light (200 µmol photone m^2 s^{-1}) for 24-hour illumination using fluorescent lamps (Figure 1). Initial cell density was 2.68 × 10^5 cells mL^{-1}. Cell counting was done every three days using a hemocytometer under a light microscope. A known volume of the culture (typically 10 µL) was loaded onto the hemocytometer chamber, and cells within the designated grid area were counted. This number was then used to calculate the total number of cells per milliliter by applying the standard hemocytometer formula, taking into account the chamber volume and dilution factor.

Dry weight for biomass measurement was obtained by filtering 5 mL of culture and drying it in an oven at 105°C for 2 hours. Cell size was measured using ImageJ software based on microscope images. Pigment extraction involved methanol treatment, vortexing, and centrifugation, with the resulting supernatant analyzed spectrophotometrically at 666 nm for chlorophyll *a* and 475 nm for total carotenoids. Pigment concentrations were calculated using the following equations (Macıas-Sánchez, *et al*., 2005; Zou and Richmond 2000):

$$\text{Chlorophyll a } (\mu g/mL) = 13.9\ A_{666}$$

$$\text{Total carotenoids } (\mu g/mL) = 4.5\ A_{475}$$

2.3 Analysis Data

Data collected on growth, biomass, and pigment production were analyzed using IBM SPSS Statistics version 20. One-way Analysis of Variance (ANOVA) was used to determine significant differences between treatments at a 0.05 significance level. Levene's Test assessed homogeneity of variances, and Duncan's Post Hoc Test was used to compare means.

3. Results and Discussion

3.1 Results

3.1.1 Cell density

Figure 1. Layout of experimental design. The glass bottles were enriched with different AMPEP (mg L-1) concentrations in a nutrient medium.

The cell density of *C. sorokiniana* cultured at different concentrations of AMPEP in the nutrient medium is shown in Figure 2. The initial density of *C. sorokiniana* was started at 2.68 x 10^6 cells mL^{-1}, and the culture was done in triplicate. ANOVA revealed that the cell density of Groups A, B, C, D, and E was 6.29±1.94 x 10^6 cell mL^{-1}, 12.5±3.73 x 10^6 cell mL^{-1}, 15.7±1.10 x 10^6 cell mL^{-1}, 14.1±1.94 x 10^6 cell mL^{-1}, and 9.10±2.58 x 10^6 cell mL^{-1}, respectively after 24-day of culture period. Analysis of variance (ANOVA) revealed that group C was significantly higher (p<0.05) than group A.

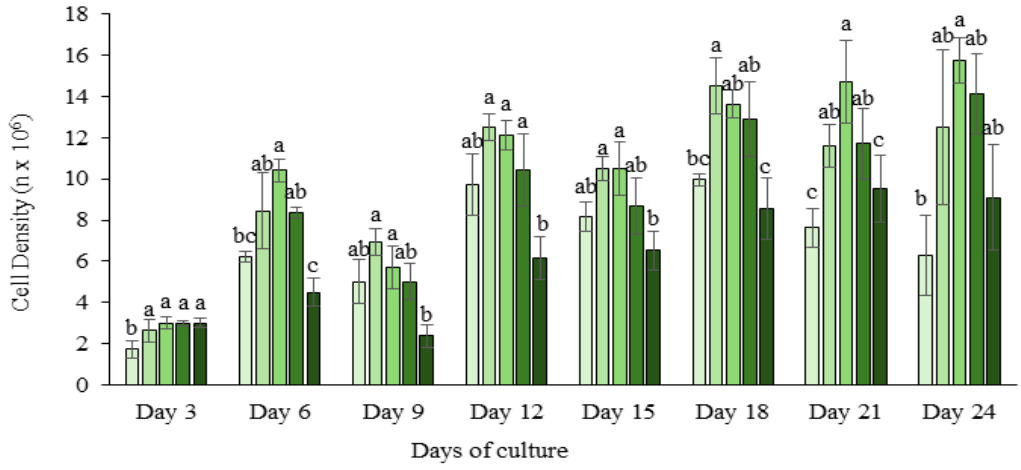

Figure 2. The graph shows the cell density (n × 10^6) of *C. sorokiniana* cultured with varying concentrations of Acadian Marine Plant Extract Powder (AMPEP). Group A (0 mg L^{-1}) served as the control, while Groups B to E received 50, 100, 150, and 200 mg L^{-1} of AMPEP, respectively. Bars that share the same letter are not significantly different from each other (p > 0.05). Data are presented as the mean ± SEM (standard error of the mean), with n = 15.

3.1.2 Specific growth rate

The specific growth rate (SGR, day^{-1}) of *C. sorokoniana* cultured at different concentrations of AMPEP in nutrient medium is shown in Figure 3. ANOVA revealed that the maximum SGR were achieved in group B (0.77±0.01 day^{-1}), group C (0.80±0.03 day^{-1}), group D (0.81±0.01 day^{-1}), and group E (0.80±0.03 day^{-1}) of which significantly different (p<0.05) than the SGR in group A (0.61±0.07 day^{-1}) as early as day 3 of culture period, however, decreases at the succeeding culture period. Moreover, Figure 4 showed that the mean SGR of group A, B, C, D, and E were 0.13±0.01 day^{-1}, 0.16±0.01 day^{-1}, 0.17±0.003 day^{-1}, 0.16±0.007 day^{-1}, 0.14±0.01 day^{-1}, respectively. ANOVA revealed that group C was significantly higher (p<0.05) than group A.

3.1.3 Cell size and biomass

The cell size (µm) of *C. sorokiniana* cultured at different concentrations of AMPEP in a nutrient medium is shown in Figure 5. The ANOVA revealed that group B (17.61±3.01 µm), group C (19.22±1.21 µm),

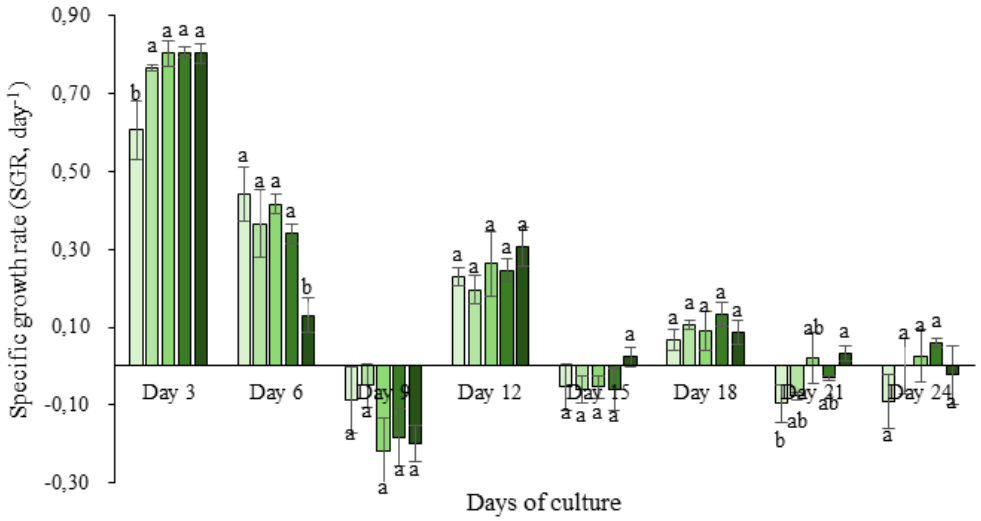

Figure 3. The graph shows the specific growth rate (SGR, day-1) of *C. sorokiniana* cultured with varying concentrations of Acadian Marine Plant Extract Powder (AMPEP). Group A (0 mg L^{-1}) served as the control, while Groups B to E received 50, 100, 150, and 200 mg L^{-1} of AMPEP, respectively. Bars that share the same letter are not significantly different from each other ($p > 0.05$). Data are presented as the mean ± SEM (standard error of the mean), with n = 15.

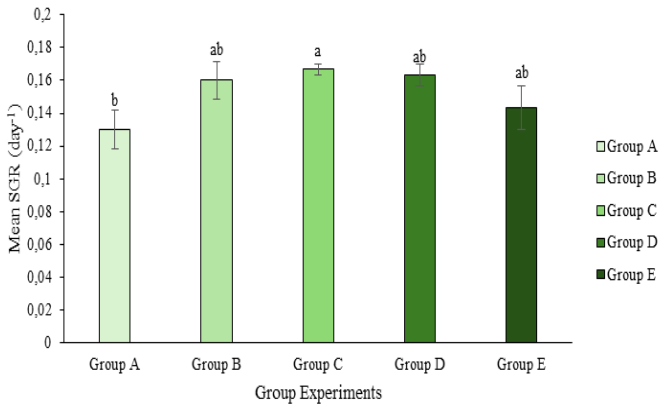

Figure 4. The graph shows the Mean SGR (day-1) of *C. sorokiniana* cultured with varying concentrations of Acadian Marine Plant Extract Powder (AMPEP). Group A (0 mg L^{-1}) served as the control, while Groups B to E received 50, 100, 150, and 200 mg L^{-1} of AMPEP, respectively. Bars that share the same letter are not significantly different from each other ($p > 0.05$). Data are presented as the mean ± SEM (standard error of the mean), with n = 15.

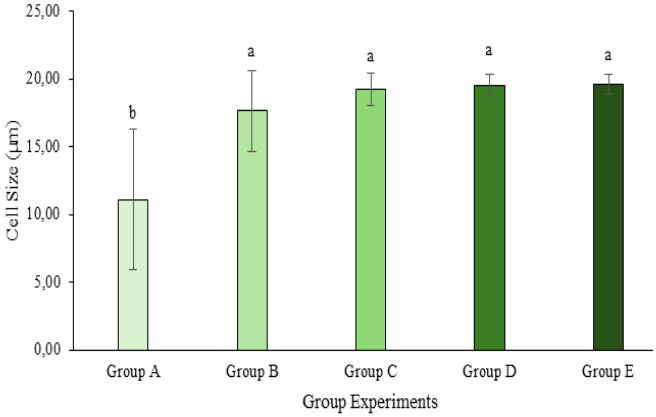

Figure 5. The graph shows the cell size (µm) of *C. sorokiniana* cultured with varying concentrations of Acadian Marine Plant Extract Powder (AMPEP). Group A (0 mg L^{-1}) served as the control, while Groups B to E received 50, 100, 150, and 200 mg L^{-1} of AMPEP, respectively. Bars that share the same letter are not significantly different from each other ($p > 0.05$). Data are presented as the mean ± SEM (standard error of the mean), with n = 15.

group D (19.51±0.77 µm) and group E (19.63±0.73 µm) of which significantly different ($p<0.05$) than the cell size of group A (11.09±5.18 µm). This indicates that the addition of AMPEP concentration in the nutrient medium increases the cell size of *C. sorokiniana* culture in all groups. Figure 6 shows the biomass (g L^{-1}) of *C. sorokiniana* cultured at different concentrations of AMPEP in the nutrient medium. ANOVA revealed that the biomass of group A, group B, group C, group D and Group E were 3.91±0.52 g L^{-1}, 6.41±0.49 g L^{-1}, 4.39±0.86 g L^{-1}, 4.59±0.96 g L^{-1}, 3.49±0.96 g L^{-1}, respectively. ANOVA revealed that the group B (6.41±0.49 g L^{-1}) was significantly ($p<0.05$) different than the biomass of Group E (3.49±0.96 g L^{-1}). This indicates that the addition of AM

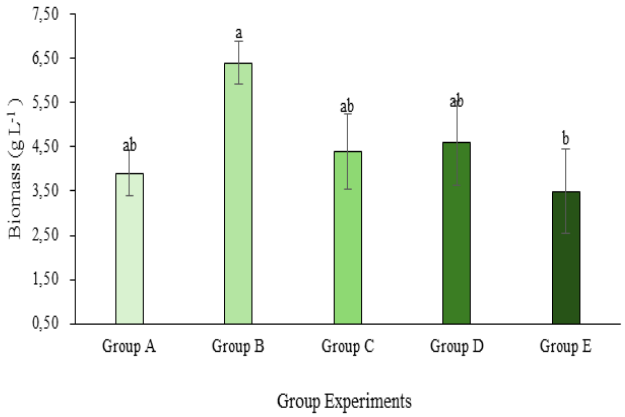

Figure 6. The graph shows the biomass (g L-1) of *C. sorokiniana* cultured with varying concentrations of Acadian Marine Plant Extract Powder (AMPEP). Group A (0 mg L^{-1}) served as the control, while Groups B to E received 50, 100, 150, and 200 mg L^{-1} of AMPEP, respectively. Bars that share the same letter are not significantly different from each other ($p > 0.05$). Data are presented as the mean ± SEM (standard error of the mean), with n = 15.

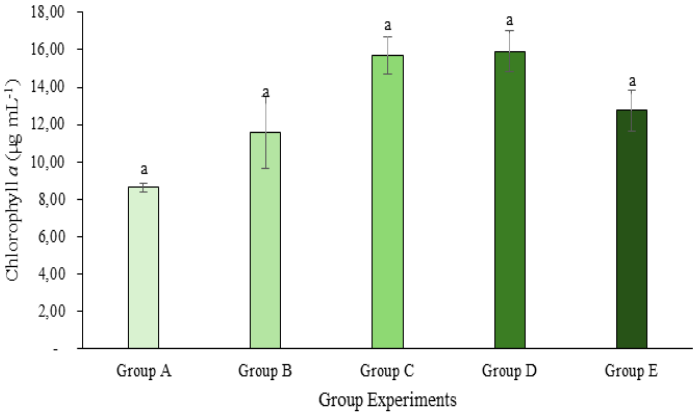

Figure 7. The graph shows the chlorophyll a pigment accumulation of *C. sorokiniana* cultured with varying concentrations of Acadian Marine Plant Extract Powder (AMPEP). Group A (0 mg L^{-1}) served as the control, while Groups B to E received 50, 100, 150, and 200 mg L^{-1} of AMPEP, respectively. Bars that share the same letter are not significantly different from each other ($p > 0.05$). Data are presented as the mean ± SEM (standard error of the mean), with n = 15.

PEP concentration significantly influences the growth of *C. sorokiniana*, with Group B achieving higher biomass than Group E.

3.1.4 Chlorophyll a pigment accumulation

The chlorophyll *a* pigment accumulation of *C. sorokiniana* cultured at different concentrations of AMPEP in a nutrient medium is shown in Figure 7. ANOVA revealed that the chlorophyll *a* pigment accumulation in group A, B, C, D, and E was 8.63±0.69 µg mL^{-1}, 11.57±5.93 µg mL^{-1}, 15.70±3.31 µg mL^{-1}, 15.92±3.76 µg mL^{-1}, and 12.77±3.42 µg mL^{-1}, respectively. Although no significant differences (p>0.05) were observed among the experimental groups, the figure indicates that increasing AMPEP concentrations (groups B, C, D, and E) enhanced chlorophyll *a* pigment accumulation in *C. sorokiniana* cultures. Additionally, Figure 8 reveals that the cellular chlorophyll *a* (pg. $cell^{-1}$) pigment accumulation of groups A, B, C, D, and E were 1.71±0.62 pg. $cell^{-1}$, 0.93±0.37 pg. $cell^{-1}$, 0.99±0.18 pg. $cell^{-1}$, 1.15±0.23 pg. $cell^{-1}$, 1.41±0.05 pg. $cell^{-1}$, respectively, no significant difference were revealed in the experimental groups. The lack of significant differences may be due to high variability and the ability of *C. sorokiniana.* to regulate chlorophyll *a* production despite varying AMPEP concentrations, maintaining stable pigment levels across treatments.

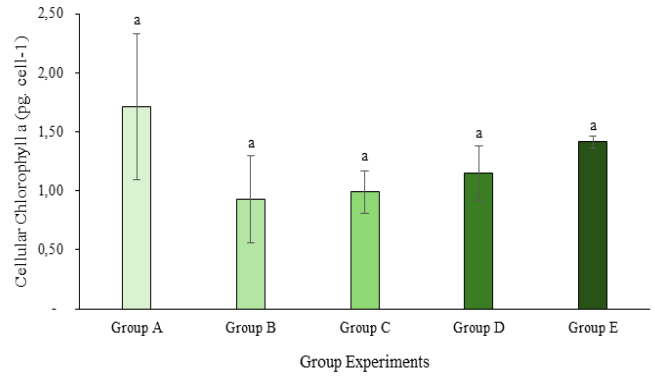

Figure 8. The graph shows the cellular chlorophyll a (pg. cell-1) pigment accumulation of *C. sorokiniana* cultured with varying concentrations of Acadian Marine Plant Extract Powder (AMPEP). Group A (0 mg L^{-1}) served as the control, while Groups B to E received 50, 100, 150, and 200 mg L^{-1} of AMPEP, respectively. Bars that share the same letter are not significantly different from each other ($p > 0.05$). Data are presented as the mean ± SEM (standard error of the mean), with n = 15.

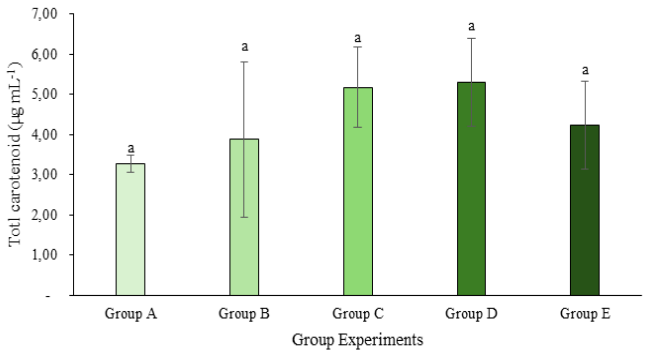

Figure 9. The graph shows the total carotenoid pigment accumulation of *C. sorokiniana* cultured with varying concentrations of Acadian Marine Plant Extract Powder (AMPEP). Group A (0 mg L^{-1}) served as the control, while Groups B to E received 50, 100, 150, and 200 mg L^{-1} of AMPEP, respectively. Bars that share the same letter are not significantly different from each other ($p > 0.05$). Data are presented as the mean ± SEM (standard error of the mean), with n = 15.

3.1.5 Total carotenoid pigment accumulation

The total carotenoid pigment accumulation of *C. sorokiniana* cultured at different concentrations of AMPEP in a nutrient medium is shown in Figure 9. Based on the results of the study, the total carotenoid pigment accumulation in groups A, B, C, D, and E was 3.28±0.21 µg mL^{-1}, 3.88±1.93 µg mL^{-1}, 5.18±1.00 µg mL^{-1}, 5.30±1.10 µg mL^{-1}, and 4.24±1.10 µg mL^{-1}, respectively. Although statistical analysis showed no significant differences ($p > 0.05$) in total carotenoid levels among the groups, the trend observed in the data suggests that increasing AMPEP concentrations may still have a biological effect. The gradual rise in carotenoid accumulation indicates that AMPEP (groups B, C, D, and E) could influence pigment biosynthesis pathways in *C. sorokiniana*, even if the variation among replicates reduced the statistical power to detect a significant difference. This suggests that while the effect is not statistically conclusive, it may still be biologically relevant and worth further investigation in future studies with more replicates or refined experimental conditions. Moreover, Figure 10 illustrates that the cellular total carotenoid pigment accumulation in groups A, B, C, D, and E was 0.65±0.23 pg. $cell^{-1}$, 0.31±0.12 pg. $cell^{-1}$, 0.32±0.05 pg. $cell^{-1}$, 0.39±0.08 pg. $cell^{-1}$, 0.47±0.02, respectively. ANOVA revealed that no significant difference ($p>0.05$) among the experimental groups.

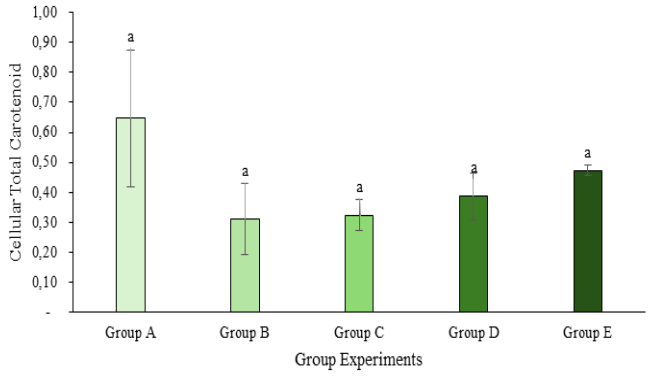

Figure 10. The graph shows the cellular total carotenoid (pg. cell-1) pigment accumulation of *C. sorokiniana* cultured with varying concentrations of Acadian Marine Plant Extract Powder (AMPEP). Group A (0 mg L^{-1}) served as the control, while Groups B to E received 50, 100, 150, and 200 mg L^{-1} of AMPEP, respectively. Bars that share the same letter are not significantly different from each other ($p > 0.05$). Data are presented as the mean ± SEM (standard error of the mean), with $n = 15$.

3.2 Discussion

3.2.1 Cell density

The present study investigated the use of AMPEP concentration in microalga *Chlorella sorokiniana* culture. According to the results of this study, AMPEP addition to the culture medium of *C. sorokiniana* culture provides higher cell densities. The higher cell density observed in Group C suggests that 100 mg L^{-1} concentration of AMPEP may be the most optimal concentration for promoting cell growth and high cell density. In contrast, Group D (150 mg L^{-1} AMPEP) and Group E (200 mg L^{-1} AMPEP) showed lower cell densities compared to Group C. This indicates that high concentrations of AMPEP may hinder growth by inducing nutrient imbalances, osmotic stress, or toxicity, as observed in studies on microalgal growth regulators (Xia *et al.*, 2021; Sarri *et al.*, 2024a). Furthermore, the brownish coloration of AMPEP may reduce light penetration in the culture medium, limiting photosynthetic efficiency. This combination of physiological stressors and diminished light availability likely contributes to the observed variations in growth performance among experimental groups (Sarri *et al.*, 2024a; Sarri *et al.*, 2024b). The significant differences in mean cell density between groups B (8.89 x 10^6 cells mL^{-1}), C (9.56 x 10^6 cells mL^{-1}), and group A (6.12 x 10^6 cells mL^{-1}) suggest that the treatments used in 50 mg L^{-1} and 100 mg L^{-1} of AMPEP concentration promote cell growth. This finding aligns with studies showing that nutrient supplements or optimized environmental conditions can enhance cell production. A study by Erbil and Durmaz (2020) found that supplementing the culture medium with 100 mg L^{-1} of myo-inositol increased cell density by 1.42-fold cell mL^{-1}, and a higher concentration of 500 mg L^{-1} resulted in a 1.28-fold increase in cell mL^{-1} compared to the control group. Similar research on microalgae growth in different media has shown significant results. For instance, Chia *et al.* (2013) reported a cell density of 2.38 x 10^6 cells mL^{-1} using varying phosphate concentrations in *Chlorella vulgaris* cultures. Additionally, Ahmad *et al.* (2016) achieved a cell density of 32 x 10^6 cells mL^{-1} using an F/2 medium with a specific phosphate source in *Chlorella* sp. cultures. Additionally, Sarri *et al.* (2024a) studied the 100 mg L^{-1} of AMPEP concentration in a nutrient medium, resulting in a 1.77-fold increase in cell mL^{-1} compared to the control group. The study demonstrates that moderate AMPEP concentrations, such as 100 mg L^{-1} of AMPEP concentration, promote optimal cell growth in *C. sorokiniana*, whereas higher concentrations may induce stress and limit growth due to factors like nutrient imbalance and reduced photosynthetic efficiency. Biostimulants like AMPEP, when applied at optimal levels, can enhance microalgal growth by stimulating physiological processes (Craigie, 2011).

3.2.2 Specific growth rate

Nutrient availability is a key factor that can influence microalgae growth (Jaiswal *et al.*, 2020). By calculating the specific growth rate (SGR) we can

identify growth-limiting factors in cell cultures. The results of this study indicated that the specific growth rate (SGR) of *Chlorella sorokiniana* was significantly enhanced by the addition of 100 mg L^{-1} concentration of AMPEP achieving the highest mean SGR of 0.17 day^{-1}, which was significantly higher than the control group. This indicates that AMPEP can effectively promote the growth of *C. sorokiniana* culture. In similar studies, particularly with *C. sorokiniana*, nutrient availability has been shown to significantly influence the specific growth rate (SGR). For instance, a comparison with the work of Erbil *et al.* (2021), who utilized BG-11 medium with *Chlorella* sp. and observed an SGR of 0.078 day^{-1}, indicating that AMPEP addition enhances the growth rate compared to standard media. Furthermore, the decline in specific growth rate (SGR) observed in this study after an initial peak is consistent with findings from several studies. For instance, a study by Dragone *et al.* (2011) found that in microalgal cultures, growth typically slows down after reaching a maximum rate due to nutrient depletion and light limitation. As the microalgae multiply and consume available nutrients, the reduced nutrient availability inhibits further cell division (Yaakob *et al.,* 2021). Similarly, Sarri *et al.* (2024b) observed that increased cell density in microalgae cultures led to self-shading, which diminished light availability for photosynthesis, contributing to a decline in growth rate. These are consistent with findings from studies on microalgal culture dynamics, where nutrient exhaustion and light limitations are primary causes for growth rate decline after initial exponential growth (Xia *et al.,* 2021; Sarri *et al.,* 2024a). Thus, this study highlights the role of nutrient availability, particularly the addition of AMPEP, in promoting the growth of *C. sorokiniana*. The observed increase in specific growth rate (SGR) with AMPEP addition demonstrates its potential as an effective growth enhancer compared to standard media. AMPEP, rich in natural plant hormones, vitamins, and micronutrients, has been shown to stimulate physiological processes and improve growth performance in both plants and microalgae (Khan *et al.,* 2009). The decline in SGR observed after the initial growth phase suggests that nutrient depletion and light limitation are key factors contributing to reduced growth rates in microalgal cultures, a phenomenon well-documented in batch culture systems (Wang *et al.,* 2008). These findings support the importance of both balanced nutrient supplementation and optimized environmental conditions in sustaining microalgal productivity.

3.2.3 Cell size

The results of cell size indicated that the addition of AMPEP to the nutrient medium leads to a significant increase in the cell size of *C. sorokiniana* culture, specifically, groups B (17.61 µm), C (19.22 µm), D (19.51 µm), and E (19.63 µm) all exhibit significantly larger cell sizes compared to the control group. This suggests that AMPEP concentrations directly influence cell growth and can promote cell enlargement. Similarly, the effects of phytohormones in previous studies, the extent of cell enlargement induced by AMPEP shows some notable differences. The optimum concentration of phytohormones (20 mg L^{-1}) resulted in significantly larger cell sizes for *C. sorokiniana*. For instance, at 20 mg L^{-}, the average cell sizes were 81.07 µm for GA3, 78.67 µm for Kinetin, 78.07 µm for IAA, and 66.90 µm for IBA (Ozioko *et al.,* 2015). This finding of the study is consistent with previous research that highlights the positive impact of fertilizers on algal cell size. For instance, the addition of nitrogen fertilizers has been shown to stimulate growth and increase the size of *C. sorokiniana* cells. Ziganshina *et al.* (2020) demonstrated that nitrogen supplementation (NH4+ or NO3−) in the culture medium led to an increase in cell size from 3.1 µm in the control group to 4.5 µm. Moreover, a study by Hadj-Romdhane *et al.* (2013), a significant increase in cell size was observed in the culture with recycled supernatant compared to the control group, the average cell size of *C. vulgaris* grown in the recycled medium was found to be 4.8 µm, compared to 3.2 µm for the cells grown in standard media. This highlights the positive impact of nutrient availability in enhancing cell growth and size. Thus, the addition of supplements such as AMPEP, phytohormones, and nitrogen-rich fertilizers to culture media significantly enhances cell size. For instance, biostimulants like AMPEP (derived from *Ascophyllum nodosum*) contain bioactive compounds that can influence microalgal physiology (Craigie, 2011). Similarly, phytohormones such as auxins and cytokinins have been shown to regulate cell division and expansion in microalgae (Han *et al.,* 2018). Additionally, nitrogen-rich fertilizers provide essential nutrients that support cell growth and biomass accumulation (Savage *et al.,* 2020).

3.2.4 Biomass

The results demonstrated the effect of varying concentrations of AMPEP on the biomass production of *C. sorokiniana* culture. Group B, with the addition of 50 mg L^{-1} of AMPEP concentration, showed a higher biomass production (6.41 g L^{-1}) compared to both Group E, with the addition of 200 mg L^{-1} of AMPEP concentration (3.49 g L^{-1}), and the control group. This suggests that AMPEP concentration plays an important role in regulating the growth of *C. sorokiniana*, with an optimal concentration promoting higher biomass accumulation. Similarly, a study by Sarri *et al.* (2024b), reported that lower concentrations of AMPEP (125 mg L^{-1}) produced higher biomass (2.57 g L^{-1}) compared to the higher concentrations (625 mg L^{-1}) and the con-

trol groups. In addition, Durmaz and Erbil (2020) cultured the microalga *Nannochloropsis oculata* in a fiberglass-reinforced plastic panel photobioreactor enriched with f/2 medium, achieved a dry weight of 0.81 g L^{-1}. Additionally, a study reported that continuous cultures enriched with f/2 medium in helical tubular photobioreactors reached productivities of 2.02 and 3.03 g L^{-1} (Briassoulis *et al.*,2010) The result of their study is similar with the present study, where lower AMPEP concentrations in the nutrient medium led to an increase in the dry weight of *C. sorokiniana* cultures. Additionally, this study exceeded the findings of Feng *et al.* (2012), who cultivated microalga *Chlorella zofingiensis* on BG-11 medium (enriched with nitrogen and phosphate) and achieved a dry weight of 0.90 g L^{-1}. Hence, the results of this study demonstrated that optimal concentrations of AMPEP such as 50 mg L^{-1} significantly enhance the biomass and dry weight production of *C. sorokiniana* culture.

3.2.5 Chlorophyll a pigment accumulation

Chlorophyll *a* is a key pigment in microalgae, playing an essential role in photosynthesis by absorbing light energy and converting it into chemical energy, which is critical for the growth and metabolism of plants and algae (Zhou *et al.,* 2019). Additionally, chlorophyll *a* has gained attention for its health-promoting properties as it acts as a potent antioxidant, helping to neutralize free radicals and protect cells from oxidative damage, which may reduce the risk of chronic diseases like cancer and cardiovascular conditions (Zhang *et al.,* 2024). Similar studies stated that chlorophyll *a* also contributes as natural pigments that offer a range of advantages, such as improving a product's visual appeal, enhancing its antioxidant activity, and extending its shelf life (Kumara *et al.,* 2017; Sun *et al.,* 2023). In the current study, chlorophyll *a* pigment accumulation increases with higher AMPEP concentrations, with group D showing the highest value (15.92 µg mL^{-1}) with 150 mg L^{-1} of AMPEP concentration. These findings are consistent with other studies, for example, a study by Ak (2012), where various organic fertilizers were tested for their impact on chlorophyll *a* accumulation in *Spirulina platensis* cultures. After 5 days, the experimental groups showed slight increases in chlorophyll *a*, with concentrations of 2.45, 1.56, 1.90, 0.67, and 0.63 mg L^{-1}, compared to an initial concentration of 0.50 mg L^{-1} of agricultural organic fertilizer. In contrast, Sarri and Elp (2024) found that nutrient media with higher iron and lower phosphate concentrations supported greater cellular pigment accumulation, with values reaching 1.378 pg $cell^{-1}$. In addition, Sarri *et al.* (2024a) studied the 150 mg L^{-1} of AMPEP concentration in a nutrient medium, resulting an increased chlorophyll *a* level of 10.89 µg mL^{-1}, compared to the control group. Thus, the findings of this study highlight the positive effect of AMPEP concentrations on chlorophyll *a* accumulation in *C. sorokiniana* culture, with higher concentrations such as 150 mg L^{-1} of AMPEP, leading to increased pigment accumulation.

3.2.6 Carotenoids pigment accumulation

Carotenoids are a group of important pigments in microalgae, known for their vital roles in photosynthesis, antioxidant activity, and contributing to the nutritional value of algae. (Wang *et al.,* 2020). Carotenoids also serve as precursors for vitamin A and possess potent antioxidant properties that protect cells from damage caused by free radicals, thereby reducing the risk of chronic diseases such as cancer and cardiovascular conditions (Liu *et al.,* 2021). Furthermore, carotenoids in microalgae have gained attention for their commercial applications in the food, cosmetic, and pharmaceutical industries due to their health-promoting effects and natural colorant properties (Saini *et al.,* 2020). In addition to their direct benefits to the algae themselves, carotenoids contribute significantly to the economic value of microalgae as a sustainable source of bioactive compounds (Zhou *et al.,* 2019). The current study demonstrated the effect of AMPEP concentrations on carotenoid pigment accumulation in *C. sorokiniana* culture, where Group D (5.18 µg mL^{-1}) showed the highest accumulation with a 150 mg L^{-1} concentration of AMPEP. Moreover, a study by Ogbonna *et al.* (2021) reported that the carotenoid accumulation from *Chlorella sorokiniana* and *Ankistrodesmus falcatus* cultured in Bold's Basal Medium (BBM) increased progressively with cultivation time. Similar studies stated that an increase in carotenoid accumulation in *Isochrysis* sp. cultured in f/2 medium, an enriched seawater medium commonly used for microalgae (Gómez-Loredo *et al.*, 2016). Additionally, Sarri *et al.* (2024a) studied the 25 mg L^{-1} of AMPEP concentration in a nutrient medium, resulting an increased total carotenoid level of 3.12 µg mL^{-1}, compared to the control group. Hence, the study indicates that AMPEP concentrations enhance carotenoid pigment accumulation in *C. sorokiniana* culture with the highest levels achieved at 150 mg L^{-1} of AMPEP concentration. These results emphasize the potential of AMPEP to enhance carotenoid pigment accumulation.

4. Conclusion

This study investigated the effect of varying AMPEP concentrations on the cultivation of *C. sorokiniana*. As a result, the optimal concentration for promoting growth, as indicated by the highest cell density, specific growth rate, and biomass, was 100 mg L^{-1} of AMPEP. The addition of AMPEP to the nutrient medium also promoted an increase in cell size, with the

largest cells observed at concentrations of 100 mg L^{-1}. However, higher concentrations such as 150 mg L^{-1} and 200 mg L^{-1} of AMPEP led to reduced cell densities, likely due to nutrient imbalances and light limitations. In pigment accumulation, the highest levels of chlorophyll *a* and total carotenoid were observed at 150 mg L^{-1} of AMPEP. This highlights that while 100 mg L^{-1} is optimal for growth and biomass production, 150 mg L^{-1} enhances pigment accumulation. Overall, 100 mg L^{-1} of AMPEP is considered the best concentration, as it provides a balance between optimal growth performance and acceptable pigment yield. Hence, the addition of AMPEP concentration in microalga *C. sorokiniana* culture may improve growth and pigment accumulation.

Acknowledgement

This paper is the undergraduate thesis of the primary author. We extend our gratitude to Mindanao State University Tawi-Tawi College of Technology and Oceanography (MSU-TCTO).

Authors' Contributions

J-na and Mar: Data Curation, Analysis, Interpretation of the Data, Writing - Original Draft; Sar: Conceptualization, Data Curation, Analysis and Interpretation of the Data, Writing - Original Draft, Writing - Review & Editing; Riz: Writing - Review & Editing; Wah: Writing - Review & Editing. All authors approved the final manuscript.

Conflict of Interest

The authors declare no conflict of interest.

Declaration of Artificial Intelligence (AI)

The author(s) affirm that no artificial intelligence (AI) tools, services, or technologies were employed in the creation, editing, or refinement of this manuscript. All content presented is the result of the independent intellectual efforts of the author(s), ensuring originality and integrity.

Funding Information

No financial support was received in this study

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