International Journal of Life Science and Agriculture Research
ISSN (Print): 2833-2091, ISSN (Online): 2833-2105
Volume 05 Issue 03 March 2026
DOI: https://doi.org/10.55677/ijlsar/V05I03Y2026-03
Impact Factor: 7.88 , Page No : 131-139

Optimizing Nutrient Media for Growth and Biomass Production of the Diatom *Thalassiosira weissflogii*

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ABSTRACT

Background: Aquaculture, particularly shrimp farming, has become an important economic sector in Viet Nam. The microalga *Thalassiosira weissflogii* is a valuable natural feed due to its high nutritional value and benefits for larval growth and water quality. Optimizing the culture medium is essential for enhancing biomass productivity and nutritional quality of this diatom for aquaculture applications.

Objectives: This study aimed to identify the optimal nutrient medium for maximizing growth, biomass yield, and nutritional quality of the diatom Thalassiosira weissflogii. The comparative evaluation of different nutrient media was undertaken to provide a scientific basis for recommending a suitable medium for large-scale microalgal biomass production in aquaculture.

Methods: The experiment involved culturing *Thalassiosira weissflogii* in four different nutrient media—AGP, Walne, F/2, and TT3—over an 8-day period, with three replicates per treatment. After cultivation, the algal biomass was analyzed for key nutritional components, including carbohydrates, carotenoids, and silica, to assess the effects of different nutrient media on algal nutritional quality.

Results: The results revealed statistically significant differences among treatments ($p < 0.05$). The AGP medium exhibited superior performance, achieving the highest growth rate, maximum cell density (30×10^8 cells/mL on day 7), and the greatest mean biomass yield (3.21 ± 0.41 g/L). In terms of nutritional composition, AGP also produced the highest levels of carbohydrates (2.4 mg/0.1 g algae), carotenoids (0.68 µg/L), and silica (22.2%) compared with the other treatments. The Walne, F/2, and TT3 media resulted in lower growth performance and nutrient accumulation.

Conclusion: The study demonstrates that the AGP medium is the optimal choice for culturing *Thalassiosira weissflogii*, meeting both high biomass productivity and superior nutritional quality requirements. This medium is therefore well suited for the production of natural live feed for seed production and commercial aquaculture.

KEYWORDS: Biomass, Cell density, Nutrient media, AGP, Walne, TT3, f/2 medium, *Thalassiosira weissflogii*

Published Online: February 25, 2026

Cite the Article: Nguyen, P.T.B., Nhu, H., Linh, N.T.T., Qui, N.H. (2026). Optimizing Nutrient Media for Growth and Biomass Production of the Diatom Thalassiosira weissflogii. International Journal of Life Science and Agriculture Research, 5(3), 131-139. https://doi.org/10.55677/ijlsar/V05I03Y2026-03

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1.0 INTRODUCTION

In recent years, aquaculture—particularly commercial shrimp farming—has expanded rapidly and become one of Vietnam's key economic sectors. Recent statistics indicate that shrimp exports reached over USD 686 million in the first quarter of 2024, a 14% increase compared to the same period in 2023, indicating a strong recovery after previous challenges involving market fluctuations and disease outbreaks (VASEP, 2025). Among various factors affecting shrimp farming, nutrition plays a pivotal role in larval survival, growth, and quality. In this context, microalgae serve as an essential live feed source (Tuyen NX, 2023). *Thalassiosira weissflogii*, a diatom species, has been recognized for its high nutritional value, particularly its rich content of essential polyunsaturated fatty acids such as DHA and EPA (7.2 mg/mL), which are critical for early-stage development in aquatic organisms (Pratoomyot, J et al.,2005). This species has demonstrated positive effects on survival, growth performance, and immune response in shrimp, mollusks, and marine fish, while also contributing to improved pond ecology (Silveira Júnior, et al., 2019).

Additionally, *T. weissflogii* has the capacity to absorb ammonia and nitrate, thereby enhancing water quality, suppressing harmful algal growth, and maintaining an optimal light-yellow water color that supports feeding behavior and reduces light stratification (Fernandes, L et al., 2019; Tam LV et al, 2020). Numerous studies in Vietnam and globally have emphasized the species' importance in hatchery systems, particularly for *Litopenaeus vannamei*, due to its high biomass productivity under laboratory conditions (Cong NV et al.,2012, Cong NV et al., 2016). According to Finkel ZV et al. (2009), deficiencies in trace elements (Fe, Cu, Zn, Co, Mn, Mo) and B-group vitamins such as B1, B6, B12, and biotin in artificial culture media can significantly reduce the growth rate and biomass productivity of microalgae. Various types of artificial nutrient media have been used in microalgal cultivation studies, including ESAW, AGP, Walne, TT3, f/2, Conway, BG-11, and Chu-10. Numerous studies have confirmed that both the composition and concentration of nutrients in the culture medium have a significant impact on the growth rate, biomass yield, and biochemical composition of *T. weissflogii* (Brzezinski, M. A, 1985).

Therefore, this study aims to optimize the nutrient composition of culture media to enhance biomass production efficiency and improve the nutritional quality of *Thalassiosira weissflogii*, thereby providing a sustainable, high-quality live feed source to support hatchery operations and commercial shrimp farming systems.

2.0 MATERIALS AND METHODS

2.1. Study Location and Experimental Period

The experiment was conducted from July to August 2024 at the Microalgae Laboratory, Faculty of Agriculture and Aquaculture, Tra Vinh University, Viet Nam.

2.2. Seawater Preparation and Starter Culture Quality

Seawater was collected from a shrimp hatchery where it had undergone preliminary filtration. The seawater was further sterilized by autoclaving at 121 °C for 15 min, cooled to room temperature, and used as the culture medium for microalgal cultivation.
Prior to inoculation, the starter culture of *Thalassiosira weissflogii* was examined to ensure culture quality. Only cultures with uniform cell morphology, consistent cell size, and healthy appearance—without visible deformities or contamination by protozoa or other algal species—were used for the experiment.

2.3. Experimental Design and Culture Conditions

Thalassiosira weissflogii was cultured in 10-L plastic tanks containing 5 L of sterilized seawater. Four nutrient media were tested, each with three replicates:

- NT1 (AGP medium): 10 mL AGP nutrient solution (20%), 500 µL silicate, 5% (v/v) inoculum
- NT2 (Walne medium): 500 µL Walne solution, 500 µL silicate, 50 µL vitamin mix, 5% (v/v) inoculum
- NT3 (F/2 medium): 10 mL F/2 nutrient solution, 500 µL silicate, 50 µL vitamin mix, 5% (v/v) inoculum
- NT4 (TT3 medium): TT3 nutrient solution (3:1 ratio), 500 µL silicate, 50 µL vitamin mix, 5% (v/v) inoculum

All cultures were maintained under controlled conditions: temperature 20–22 °C; salinity 28–30‰; light intensity approximately 3000 lux; continuous illumination (24 h light); and continuous aeration. The initial inoculum density was standardized across treatments.

2.4. Growth Monitoring and Cell Density Measurement

Algal growth was monitored daily for 8 days. Cell density was estimated using the optical density (OD) method measured with a spectrophotometer at 680 nm. This approach is widely used for rapid monitoring of microalgal and diatom growth in laboratory cultures (Guillard & Ryther, 1962; Becker, 2013). The OD values were calibrated using a standard curve relating OD to cell density for *Thalassiosira weissflogii* to ensure measurement accuracy.

2.5. Biomass Harvesting

At the end of the cultivation period, algal biomass was harvested by centrifugation at 4000 rpm for 15 min at room temperature (~25 °C). The harvested biomass was collected, dried, and weighed to determine biomass yield (g/ L).

2.6. Biochemical Composition Analysis

2.6.1. Carbohydrate Content

Total carbohydrate content was determined using the phenol–sulfuric acid method. Briefly, algal samples were reacted with phenol and concentrated sulfuric acid, and absorbance was measured at 490 nm. A glucose standard curve was used to calculate carbohydrate concentration, which was expressed as mg per 0.1 g dry biomass.

2.6.2. Carotenoid Content

Total carotenoid content was quantified following the method of Hoang Thi Lan Anh et al. (2010) with minor modifications. Algal biomass was homogenized with glass beads and extracted using cold 90% acetone. The extract was filtered and adjusted to a final volume of 10 mL. Absorbance was measured at 480 nm, and carotenoid concentration was calculated according to Strickland and Parsons (1972), expressed as µg/ L.

Pigment content (µg/ L) = C/V

Where:

- V is the volume of the algal sample filtered (L).
- C (total carotenoids) = 4.0 x E_{480} (mg).
- E_{480} is the optical density (OD) value measured at a wavelength of 480 nm.

2.6.3. Biosilica Content

Biosilica content was determined following the method described by Qi et al. (2016). Dried algal biomass was subjected to acid washing using dilute hydrochloric acid (~2% HCl) to remove mineral impurities, followed by rinsing with distilled water. The treated biomass was then calcined at approximately 600 °C to remove organic matter and obtain purified biosilica.

Silica content was calculated gravimetrically and expressed as the percentage of SiO_2 relative to the dry biomass weight.

The percentage of silica (SiO_2) was determined using the following equation:

$$\%SiO_2 = \frac{m_{processed}}{m_{total}} \times 100\%$$

Where: $m_{processed}$ is the mass of SiO_2 obtained after acid treatment and calcination, and m_{total} is the total mass of the initial product

2.7. Statistical Analysis

All data were expressed as mean ± standard deviation. Statistical analyses were performed using SPSS version 20. One-way analysis of variance (ANOVA) followed by Duncan's multiple range test was applied to determine significant differences among treatments at $p < 0.05$.

3.0 RESULTS AND DISCUSSION

3.1. Growth Performance of *Thalassiosira weissflogii*

The growth performance of Thalassiosira weissflogii cultured in four nutrient media (AGP, Walne, F/2, and TT3) over an 8-day period is shown in Figure 3.1. In all treatments, cell density increased from day 0 and reached a maximum between days 6 and 7, followed by a slight decline on day 8, indicating a transition from the exponential to the stationary growth phase. This growth pattern is typical for batch cultures of marine diatoms under controlled laboratory conditions (Brzezinski, 1985; Guillard & Ryther, 1962).

Significant differences in growth were observed among treatments ($p < 0.05$). The AGP medium supported the highest cell density, reaching approximately 30 × 10^8 cells/mL on day 7, whereas Walne medium resulted in moderate growth. In contrast, F/2 and TT3 media exhibited lower cell densities throughout the culture period. Notably, cultures grown in TT3 peaked earlier and declined rapidly, suggesting nutrient limitation during the later stages of cultivation. These results are consistent with previous findings showing that TT3 medium yields lower growth rates in *Nannochloropsis oculata* compared to Walne and F/2 (Thuy NTT et al, 2021).

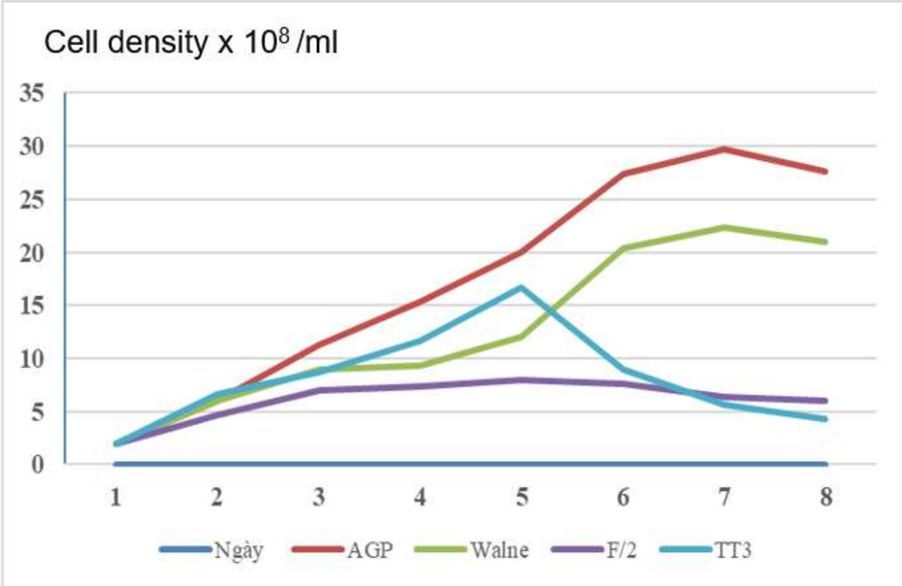

Figure 3.1. Cell density of *Thalassiosira weissflogii* cultured in four different media: AGP, Walne, F/2, and TT3

The observed differences are attributed to nutrient composition. While all media supply nitrogen and phosphorus, TT3 utilizes KNO_3 as the nitrogen source, whereas Walne and F/2 use $NaNO_3$. Additionally, TT3 lacks essential trace elements and vitamins necessary for optimal algal metabolism (Cong NV and Duong NK, 2014).

AGP's superior performance can be explained by its more balanced nutrient formulation, including ferric ions (Fe^{3+}) crucial for photosynthesis. The combination of nitrate, phosphate, silicate, and trace metals in AGP likely enhances protein synthesis, cell division, and overall biomass accumulation. These findings align with prior studies reporting enhanced growth of *T. weissflogii* in AGP20% medium, with peak densities observed on day 6–7 (Thuy NTT et al, 2021). Nguyen Thi Thanh Tuyen (2020), also identified AGP as the most effective medium for this species. Furthermore, Nguyen Van Minh et al. (2018) noted that photosynthetic efficiency may decline at high cell densities due to self-shading—consistent with the minor decline observed after day 7.

Collectively, the data confirm that AGP is the most favorable medium for promoting high-density cultivation of *T. weissflogii*, followed by Walne, whereas F/2 and TT3 are less suitable under the tested conditions.

3.2. Algal Biomass Harvested by Centrifugation

Biomass yields obtained after centrifugation are presented in Figure 3.2. Although no statistically significant differences were detected among treatments ($p > 0.05$), clear trends were evident. The highest mean biomass yield was recorded in the AGP medium (3.21 ± 0.41 g/L), followed by Walne, F/2, and TT3.

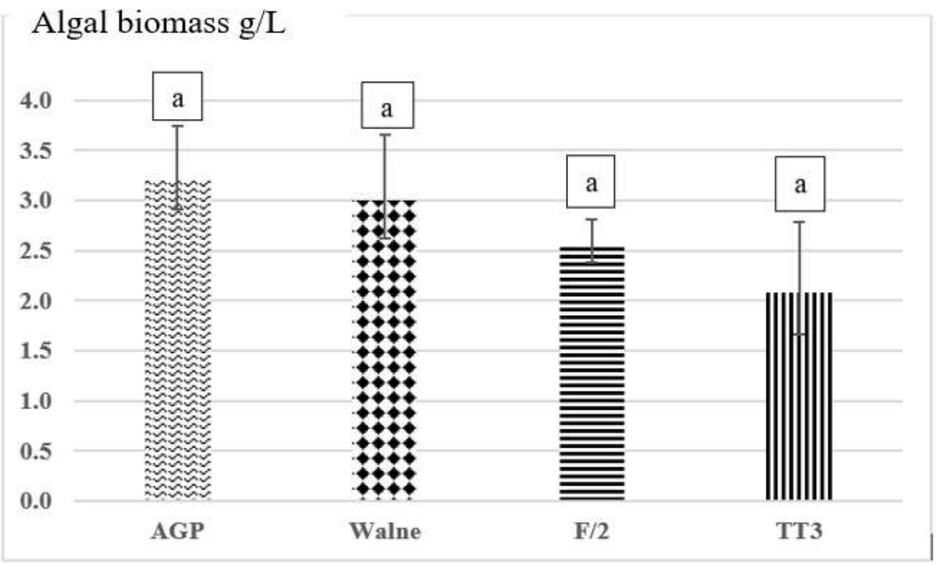

Figure 3.2. Biomass yield of *Thalassiosira weissflogii* cultured in four different media

Note: The letter "a" above each column indicates that the values are not significantly different ($p > 0.05$).

AGP's superior performance may be attributed to its enriched formulation, containing not only macronutrients but also growth-promoting compounds such as B-group vitamins and trace metals (Fe, Mn, Zn, Cu), which are essential for photosynthesis and cell division (Sánchez-Bayo, A et al, 2024). This is consistent with findings by Abdelhay et al. (2025), who reported enhanced biomass in diatoms following nutrient optimization and vitamin supplementation.

The relatively lower yield in TT3 aligns with prior observations that T/TT-based media, while suitable for optimizing biochemical profiles, are less effective for biomass production (Sulaiman Madyod et al, 2025). In contrast, AGP and Walne provide a balanced nutrient profile that supports both growth and cell proliferation.

Overall, AGP and Walne represent suitable media for cultivating *T. weissflogii* when high biomass yield is the primary objective. These findings underscore the critical role of nutrient composition in microalgal productivity and offer practical insights for applications in aquaculture and bioresource development.

3.3. Results of Biosilica Extraction from *Thalassiosira weissflogii*

The biosilica content of *T. weissflogii* cultured in different nutrient media is shown in Figure 3.3. Significant differences among treatments were observed ($p < 0.05$), with the highest silica content recorded in AGP (22.2% dry weight), followed by F/2 (20.4%), Walne, and TT3 (13.2%).

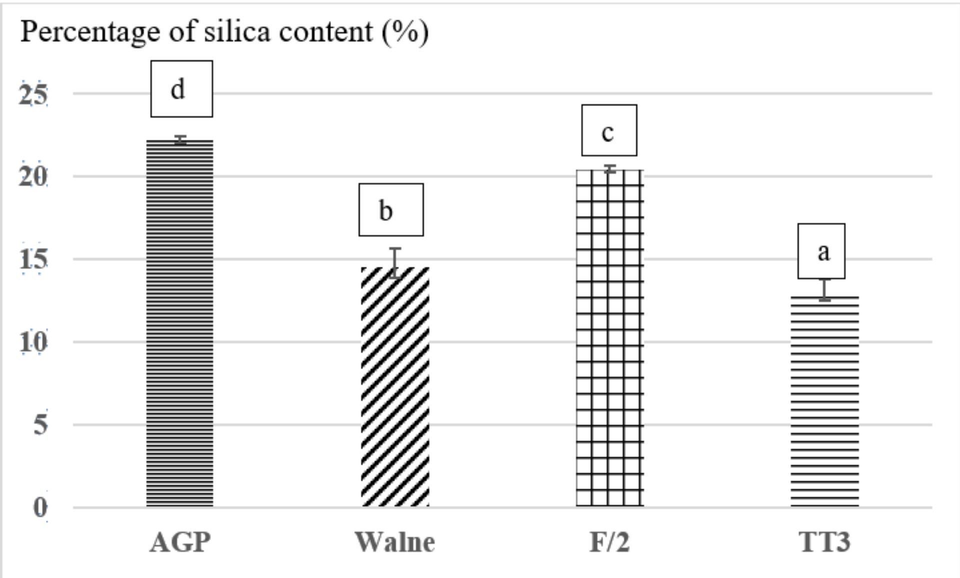

Figure 3.3. Biosilica yield of *Thalassiosira weissflogii* cultured in four different media

Note: Different letters "a, b, c, d" above each column indicate statistically significant differences ($p < 0.05$).

AGP exhibited the highest silica accumulation (22.2%), suggesting its superior silicate availability for frustule biosynthesis. This aligns with reports that commercial nutrient supplements often include sodium silicate to promote silicification (Guillard et al, 1962). F/2 medium, known for its silicate stability, yielded 20.4%, consistent with prior observations in *Chaetoceros* spp. (18–22%) (Lobus, N. V, 2024). In contrast, Walne and TT3 recorded lower silica levels, likely due to suboptimal silicate content or Si:N ratios, echoing previous findings that Walne is more suitable for non-diatom species (FAO, 2020). Silicate availability and its balance with nitrogen are critical in regulating diatom silicification. As shown by Yi, Z et al (2019), excess silicate promotes silica accumulation, whereas limitation suppresses frustule formation despite continued growth. The low silica content in TT3 supports this, indicating poor silicate provision.

All treatments fell within the typical silica range for diatoms (10–40% DW) (Nelson, D. M et al, 1995), confirming the validity of results. Overall, the study underscores the importance of silicate optimization in culture media to enhance biosilica yield, particularly for applications in seed production and aquaculture.

3.4. Carotenoid Extraction Results in *Thalassiosira weissflogii*

Carotenoid content differed significantly among nutrient media ($p < 0.05$), as illustrated in Figure 3.4. The highest carotenoid concentration was observed in AGP (0.68 µg/ L), followed by F/2, Walne, and TT3.

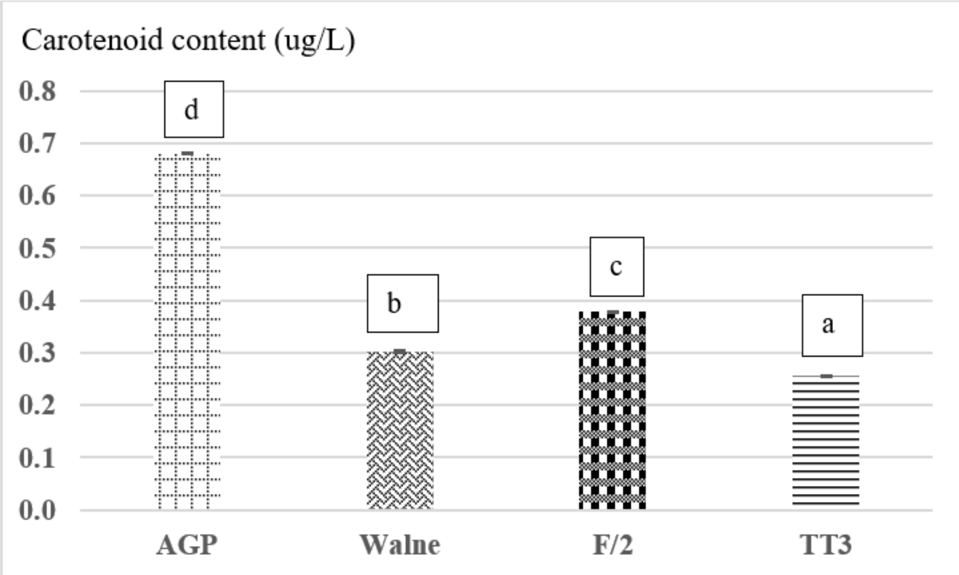

Figure 3.4. Carotenoid content of *Thalassiosira weissflogii* cultured in four different media

Note: Different letters "a, b, c, d" above each column indicate statistically significant differences ($p < 0.05$).

These variations are attributed to differences in nitrate, phosphate, and trace metal availability, which regulate carotenoid biosynthesis and photosynthetic efficiency (Mischler, J. A et al, 2014). F/2 medium supported relatively high pigment production, consistent with earlier studies reporting 0.35–0.45 µg/L in *Chaetoceros* spp. (Sipaúba-Tavares et al, 2017) , likely due to its balanced nitrate levels and sufficient micronutrients (Guillard et al, 1962). In contrast, the lower N:P ratio in Walne medium may constrain pigment synthesis, as also reported by López et al., (2020). AGP yielded the highest carotenoid content, suggesting enhanced nutrient supply for pigment production. This aligns with Braga et al. (2020), who showed that nitrogen- and micronutrient-rich media boost carotenoid levels by 30–50% in diatoms. The lowest carotenoid concentration in TT3 further reflects its nutritional limitations, possibly suppressing enzyme activity related to pigment synthesis (Paliwal, C et al, 2017).

Overall, the carotenoid values obtained (0.25–0.70 µg/L) fall within the expected range for diatoms under laboratory conditions (0.20–0.80 µg/L) (Matos, A. P et al, 2019), affirming the physiological relevance and reliability of the results.

3.5. Carbohydrate Content in *Thalassiosira weissflogii*

Carbohydrate content in *T. weissflogii* was quantified using the phenol–sulfuric acid method, with results calculated from a standard calibration curve. Significant variation in carbohydrate accumulation was observed among the four media tested (Figure 3.5). The AGP medium yielded the highest carbohydrate content (2.4 mg/0.1 g biomass).

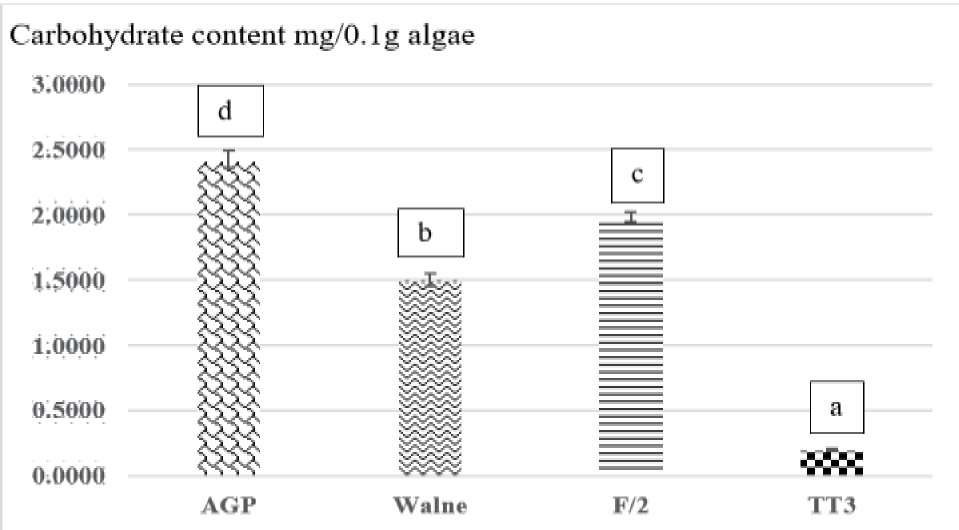

Figure 3.5. Carbohydrate content of *Thalassiosira weissflogii* cultured in four different media

Note: Different letters "a, b, c, d" above each column indicate statistically significant differences ($p < 0.05$).

These differences are attributed to the availability of nitrate, phosphate, and micronutrients essential for photosynthesis and carbohydrate biosynthesis. This aligns with Becker (2013), who reported that nutrient-rich media promote carbohydrate accumulation via enhanced photosynthetic activity. Similarly, Guillard & Ryther (1962) and Reynolds (2006) noted that F/2 and Walne support moderate to high carbohydrate levels depending on species and light conditions. The lower carbohydrate yield in TT3 likely reflects nitrate or trace element limitations (Fábregas, J et al., 1989).

The superior performance of AGP is consistent with findings by Chu et al. (2019), who highlighted that organic- or nutrient-balanced media enhance carbohydrate production in microalgae. These results suggest that AGP is an effective medium for cultivating *T. weissflogii* when aiming for carbohydrate-rich biomass applicable to aquaculture or bioproduct development.

V. CONCLUSION

This study demonstrates that nutrient medium composition plays a critical role in regulating the growth performance, biomass productivity, and biochemical composition of the marine diatom *Thalassiosira weissflogii*. Among the four nutrient media evaluated, AGP consistently outperformed Walne, F/2, and TT3 across all assessed parameters, including cell density, harvested biomass, biosilica content, carotenoid concentration, and carbohydrate accumulation.

Specifically, AGP supported the highest cell density (30×10^8 cells/mL), biomass yield (3.21 ± 0.41 g/L), silica content (22.2% dry weight), carotenoid concentration (0.68 µg/L), and carbohydrate content (2.4 mg/0.1 g biomass). These results highlight the effectiveness of AGP in providing a balanced supply of macronutrients, silicate, trace elements, and vitamins required for optimal diatom growth and metabolic activity. Walne medium showed moderate performance, while F/2 and TT3 were less suitable under the experimental conditions, likely due to nutrient limitations.

Overall, AGP is identified as the most appropriate nutrient medium for cultivating *T. weissflogii* for aquaculture applications, particularly in shrimp hatchery systems where high-quality live feed is required. Future studies should focus on scaling up cultivation systems and further optimizing environmental factors such as light intensity, salinity, and initial inoculum density to enhance productivity under commercial conditions.

Ethical approval
Authors declare that there are no ethical issues involved in the conduct of the study.

Conflict of Interests
The authors declare they have no competing interests, be it financial or personal interests, that could have appeared to influence the outcome of the results presented in this paper.

Funding statement
The research was funded by Tra Vinh University, Vietnam

Author contribution
Pham Thi Binh Nguyen: Conceptualization, Research project administration, Supervision, Methodology, Data analysis, Writing – original draft, Writing – review & editing. Responsible for developing the research idea, preparing the research proposal, managing and supervising the implementation of the study, evaluating research outcomes, analyzing data, and finalizing the manuscript in both Vietnamese and English.
Huynh Nhu: Investigation, Experimentation, Data collection. Conducted the research experiments and provided the raw data for the study.
Nguyen Thi Truc Linh: Writing – review & editing. Reviewed the manuscript and edited the English version.
Nguyen Hoang Qui: Resources, Writing – review & editing, Formatting. Provided journal information and edited the English manuscript according to the journal's formatting requirements.
All authors contributed to the conceptualization, methodology design, interpretation of results, approved the final manuscript, and agree to be accountable for the research.

Acknowledgement
We acknowledge the support of time and facilities from Tra Vinh University (TVU) for this study

Data Availability: Data will be made available based on request.

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