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EFFECT OF DIFFERENT SUBSTRATES ON BIOFILM GROWTH AND LIPID CONTENT OF DIATOM *Thalassiosira sp.*

Joan T. Tabaquirao[*)], Cleresa S. Dionela[*)], Fredson H. Huervana[*) #], and Rex Ferdinand M. Traifalgar[*)]

[*)] Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Miagao, Iloilo, Philippines

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ABSTRACT

Diatoms are valuable as natural feed in aquaculture due to their lipid content and the presence of essential polyunsaturated fatty acids (PUFAs), including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). While traditional suspended cultivation has its limitations, attached cultivation offers advantages such as reduced water use and enhanced lipid productivity. This study evaluated the growth, biomass, and lipid content of Thalassiosira sp. grown on polycarbonate (PC) and polyvinyl chloride (PVC) as attachment substrates. The control group was cultured in the standard suspended cultivation method. Test attachment substrates were submerged in sterile seawater enriched with F-medium, and growth was monitored for four days. At day 4 of the culture, cell density was significantly higher on PC (13.08 × 10^5 cells mL^{-1}) and PVC (13.01 × 10^5 cells mL^{-1}) compared to the control (7.93 × 10^5 cells mL^{-1}). The specific growth rate was also significantly higher on both substrates, exhibiting a doubling time of 0.20 days. Biomass accumulation was highest on PC (27.47 mg 100 mL^{-1}), followed by PVC (26.87 mg 100 mL^{-1}), representing increases of 38.39% and 35.37% over the control (19.85 mg 100 mL^{-1}), respectively. Lipid content was higher in the attached culture system, reaching 8.50% on PC and 7.45% on PVC, corresponding to increases of 167.30% and 134.28% over the control (3.18%). These findings highlight the potential of PC and PVC as effective substrates for biofilm-based cultivation of Thalassiosira sp., demonstrating superior growth, biomass yield, and lipid accumulation compared to the suspended culture method.

KEYWORDS: *Thalassiosira* sp.; biofilm formation; polycarbonate; polyvinyl chloride; lipid content

INTRODUCTION

Diatoms have been widely used in aquaculture as live feed for various organisms, including shrimp larvae, abalone, and bivalve mollusks such as oysters. They possess a rich nutritional profile and can accumulate high amounts of lipids, particularly polyunsaturated fatty acids (PUFAs), including essential ù-3 fatty acids–eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Zulu *et al*., 2018). These fatty acids are crucial for fish growth, development, survival, and the prevention of physiological disorders and morphological abnormalities (Tocher & Glencross, 2015; Huervana *et al*., 2022). As the demand for ω-3 fatty acids increases, traditional sources such as fish oils are limited to meet global aquaculture needs, positioning diatoms as a promising alternative source of PUFAs. Generally, diatoms contain between 6-30% lipids (Fields & Kociolek, 2015; Joseph *et al*., 2017), but their lipid content can be significantly enhanced by modifying the environmental conditions. Various strategies have been explored to increase lipid production in diatoms, one of which is the non-suspended cultivation method.

Typically, microalgae are cultivated in a suspended state in water. However, this method has significant drawbacks, including high operational costs, high energy consumption, and lower biomass yield (Katarzyna *et al*., 2015). To address these challenges, alternative cultivation approaches have been explored. One such method is attached microalgae cultivation, where cells grow on solid surfaces instead of being suspended in water. This approach offers several advantages over traditional suspended cultivation, including reduced water requirement (Podola *et al*., 2017), cost-effective and reusable supporting substrates (Garbowski *et al*., 2017), enhanced wastewater treatment efficiency (Shen *et al*., 2018; Ma *et al*., 2018; Zhang *et al*., 2024), and simplified operational procedures (Berner *et al*., 2015; Yin *et al*., 2015; Gross *et al*., 2015). Furthermore, harvesting biomass is more efficient in the attached system, as microalgal cells can be scraped off the substrate, eliminating the need for expensive separation techniques such as filtration and centrifugation. Consequently, this

[#] Correspondence: Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Miagao, Iloilo, Philippines
E-mail: fhhuervana@up.edu.ph

method reduces overall operational costs, potentially benefiting aquaculture farmers. Earlier studies have shown that biofilm-based cultivation systems improved algal biomass productivity and lipid accumulation as compared to suspended cultivation methods (Berner *et al*., 2015; Ennaceri *et al*., 2023; Bagheri *et al*., 2024). Additionally, mixotrophic microalgal biofilm culture strategies were reported to enhance productivity and increase lipid accumulation in algal biofuel feedstocks (Roostaei *et al*., 2018; Nodque *et al*., 2024). These findings highlight the potential of attached cultivation in enhancing lipid and biomass production in microalgae.

Diatoms are among the primary colonizers of surfaces in both marine and freshwater environments (de Carvalho, 2018; Smith *et al*., 2021). Several studies have demonstrated their ability to attach to various substrates, including stainless steel (Richard *et al*., 2017), polyethylene terephthalate (PET) drinking bottles (Oberbeckmann *et al*., 2016), low-density polyethylene (LDPE), polypropylene (PP), and polystyrene (PS) (Dudek *et al*., 2020), as well as high-density polyethylene (HDPE) and biodegradable PET plastic bags (Eich *et al*., 2015). However, little is known about the biofilm-forming capacity of *Thalassiosira* sp. on different substrates. This study aims to address this gap by evaluating the effect of different substrates on the biofilm growth of *Thalassiosira* sp. Specifically, it seeks to determine the cell density, growth rate, biomass accumulation, and crude lipid content. The findings from this study could help optimize biofilm-based diatom cultivation, potentially enhancing their application as a sustainable source of lipids for aquaculture.

MATERIALS AND METHODS

Experimental design and setup

This experimental study was conducted over four days at the Multi-species Hatchery Complex of the Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas in Miagao, Iloilo (10°38'20.3"N 122°13'35.5"E). The experiment followed a completely randomized design (CRD) with three experimental groups: T1 (control-suspended), T2 (PC substrate), and T3 (PVC substrate), each with three replicates.

Microalgae and culture conditions

A stock culture of *Thalassiosira* sp. was obtained from the Southeast Asian Fisheries Development Center (SEAFDEC/AQD) Phycology Laboratory in Tigbauan, Iloilo. For the suspended method, *Thalassiosira* sp. was cultivated in a 750 mL glass bottle containing 450 mL of UV-treated and chlorinated seawater and 200 mL of algal inoculum, with an initial concentration of 4.60 x 10^5 cells mL^{-1}. For the non-suspended method, the same volume of algal stock and water was added to the culture container.

Polycarbonate (PC) and polyvinyl chloride (PVC) sheets (13.21 cm x 6.35 cm) were used as the test substrates for biofilm formation. Before use, the exposed surfaces of both substrates were roughened with a 220-grit sandpaper to enhance microalgal adhesion. These substrates were positioned at a slant inside the glass jars to maximize surface colonization and biofilm formation.

The culture water of the *Thalassiosira* sp. was enriched with F-medium (Guillard & Ryther, 1962) and was maintained at a salinity of 30 ppt and a water temperature of 25 °C. The cultures were illuminated under a light intensity of 100 μmol photons m^2 s^{-1} and provided with mild aeration at a rate of 0.2 L air min^{-1}.

Cell density monitoring and biofilm growth determination

On every sampling day, 3 mL samples were collected from triplicate suspended cultures to monitor cell density. On the fourth day, a 1 cm^2 section of *Thalassiosira* sp. biofilm was randomly scraped from the PC and PVC substrates, resuspended in 1 mL of sterile seawater, and homogenized by agitation. Algal growth was quantified manually by using a Neubauer chamber or hemocytometer under a compound microscope. The specific growth rate (μ) and doubling time (*dt*) were calculated following the formula by Bhattacharya & Shivaprakash (2005):

$$\mu = \frac{(\ln X_2 - \ln X_1)}{(t_2 - t_1)}$$

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

where: X_1 and X_2 are biomass concentrations at time intervals t_1 and t_2, ln 2 is equal to 0.693, and *m* is the specific growth rate

Algal biomass determination

The biomass was determined according to a reported method with slight modifications (Yaakob *et al*., 2021). On the fourth day of culture, when *Thalassiosira* sp. reached the stationary phase, algal cells were harvested by centrifugation at 4000 rpm, followed by vacuum filtration using a Whatman GF/C glass microfiber filter (47 mm diameter). The collected wet algal biomass was transferred to a pre-weighed

Petri dish and weighed. The samples were then oven-dried at 60 °C for 24 hours, and the dry biomass weight was determined by subtracting the weight of the empty Petri dish from the total weight of the dish containing the dried algal biomass.

Crude lipid content analysis

The crude lipid content was determined using the modified Bligh and Dyer method (1959). Dried algal biomass was first pulverized using a mortar and pestle. A 0.5 g sample was transferred to a 125 mL Erlenmeyer flask, moistened with water, and stored in the refrigerator overnight. The following day, 10 mL of chloroform and methanol (1:1, v/v) were added to the sample, which was then sonicated for one hour and stored in a refrigerator overnight. Sonication was used to disrupt the algal cell walls and release lipids.

On the third day, an additional 15 mL of chloroform and methanol (1:1, v/v) were added, and the sample was sonicated again for one hour. The algal biomass was then removed via vacuum filtration, and the filtrate was transferred to a separatory funnel. Distilled water was added until a clear upper phase was observed. The bottom organic phase was collected and transferred to a round-bottom flask for rotary evaporation. The extracted lipids were then transferred to a clean, pre-weighed vial and allowed to evaporate overnight. The dried lipid extracts were weighed to determine the crude lipid content as a percentage of dry weight.

Statistical analysis

All data were subjected to descriptive statistics and one-way analysis of variance (ANOVA) using SPSS version 22.0. Tukey's post hoc test was performed to determine significant differences between treatments at a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Effect of different substrates on biofilm growth

After four days of cultivation, *Thalassiosira* sp. exhibited an exponential growth curve, consistent with the typical growth pattern of most phytoplankton species (Figure 1). The cell density of different treatments gradually increased from day one, reaching a peak level on day four, with an average ranging from 7.93×10^5 to 13.08×10^5 cells mL^{-1}. The total cell density (suspended and substrate-associated) at peak growth is shown in Figure 2. A significant difference ($p < 0.05$) in total cell count was observed on day four, with the highest density recorded in the PC treatment (13.08×10^5 cells mL^{-1}), followed closely by PVC (13.01×10^5 cells mL^{-1}). These values were 64.95% and 64.11% higher than the control, respectively.

These findings align with Gómez-Ramírez et al. (2019), who reported that *Navicula* sp. adhered more efficiently to plastic substrates compared to wood or fabric, likely due to the biofilm-promoting properties of plastic surfaces. The same study also demonstrated that *Navicula incerta* and *Navicula* sp. formed biofilms more effectively on rough surfaces than on smooth surfaces. In this study, both PC and PVC substrates were roughened with sandpaper, creating nanoscale bumps that may have provided anchoring sites for microalgae, enhancing adhesion. While rougher textures are generally associated with greater adherence, this contradicts Tsavatopoulou and

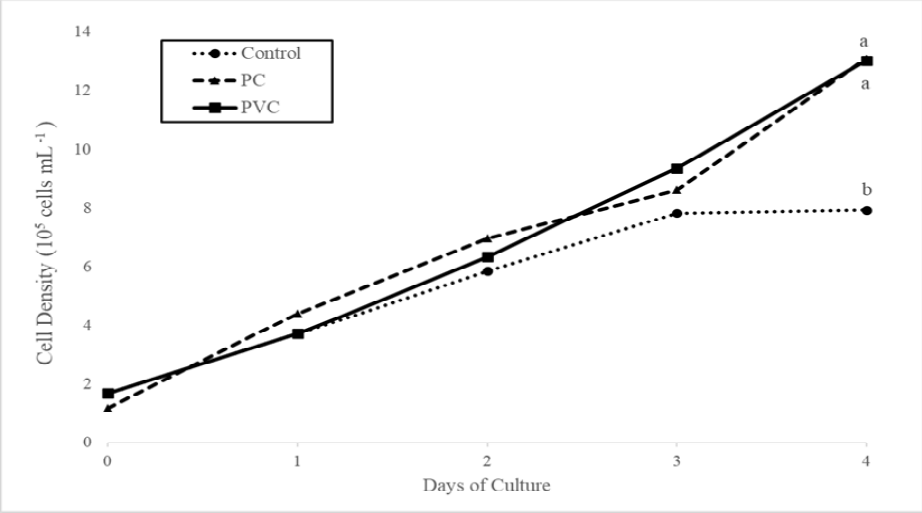

Figure 1. Growth curve of *Thalassiosira* sp. cultured under suspended and non-suspended cultivation methods. Values are means of three replicates ± SEM. Data points with different superscript letters indicate significant differences between treatments ($p < 0.05$).

Manariotis (2020), who found that microalgae exhibited higher biofilm formation on smoother surfaces, such as plexiglass and stainless steel. However, Tong & Derek (2021) reported increased diatom abundance on surfaces with rough textures, further highlighting the role of substrate characteristics in biofilm formation.

Aside from surface roughness, the high cell density of *Thalassiosira* sp. on plastic substrates may also be attributed to the availability of nutrients during peak growth, which supports exponential proliferation. Other factors influencing microalgal attachment include surface hydrophobicity and biocompatibility (von Ammon *et al*., 2018; Miao *et al*., 2020), which likely contributed to the enhanced biofilm formation observed in this study.

Table 1 shows that the specific growth rate of *Thalassiosira* sp. on both substrates was significantly higher than in the control ($p < 0.05$). Under optimal conditions, *Thalassiosira* sp. typically doubles its biomass within 8 hours. In this study, the doubling time was reduced to 4.8 hours (0.20 days) on PC and PVC substrates, while the longest doubling time was recorded in the control (without substrate).

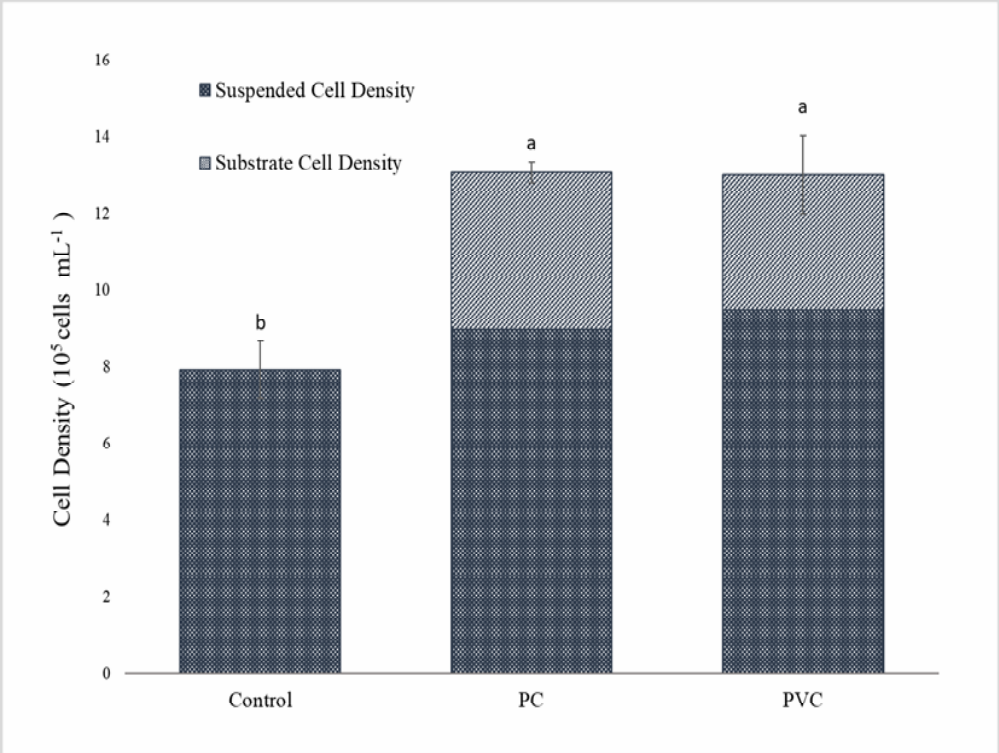

Figure 2. Total cell density (suspended + substrate-associated) of *Thalassiosira* sp. under suspended and non-suspended cultivation methods. Values are means of three replicates ± SEM. Bars with different superscript letters indicate significant differences between treatments ($p < 0.05$).

Table 1. Growth performance parameters of *Thalassiosira* sp. under suspended and non-suspended cultivation methods

	Specific growth rate (day $^{-1}$)	Doubling time (day)
Control	3.33 ± 0.03 ^{b}	0.21 ± 0.00 ^{b}
PC	3.50 ± 0.01 ^{a}	0.20 ± 0.00 ^{a}
PVC	3.48 ± 0.03 ^{a}	0.20 ± 0.00 ^{a}

Values are means of three replicates ± SEM. Different superscript letters indicate significant differences between treatments ($p < 0.05$).

Although *Thalassiosira* sp exhibited greater adherence to PC than to PVC, the difference was not statistically significant ($p > 0.05$) (Figure 2). The minimal variation in cell density between the two treatments may be due to the similar physicochemical properties of both plastic-based substrates, which influence biofilm attachment. This finding aligns with Gross *et al*. (2016), who reported that algal colonization was higher on PC than on PVC under stationary conditions.

Beyond substrate material, factors such as culture medium composition and environmental conditions, including irradiance, temperature, salinity, and nutrient availability, also play crucial roles in biofilm adherence and stability (Dang & Lovell, 2016; Miao *et al*., 2019; Qian *et al*., 2023). The results of this study demonstrate that *Thalassiosira* sp. can effectively colonize polycarbonate (PC) and polyvinyl chloride (PVC) roofing sheets. Compared to cells in suspension, biofilm-forming *Thalassiosira* sp. aggregates into colonies, resulting in increased cell density and enhanced growth rate.

Effect of different substrates on microalgal biomass

A statistically significant difference ($p < 0.05$) in the biomass of *Thalassiosira* sp. was observed among treatments on a dry weight basis (Figure 3). The highest biomass was recorded in the PC treatment (27.47 mg 100 mL^{-1}), followed closely by PVC (26.87 mg 100 mL^{-1}). Compared to the control (without a substrate), biomass yield was significantly higher by 38.39% in the PC treatment and 35.37% in the PVC treatment. These findings suggest that substrate surface properties strongly influence the biomass production of diatoms.

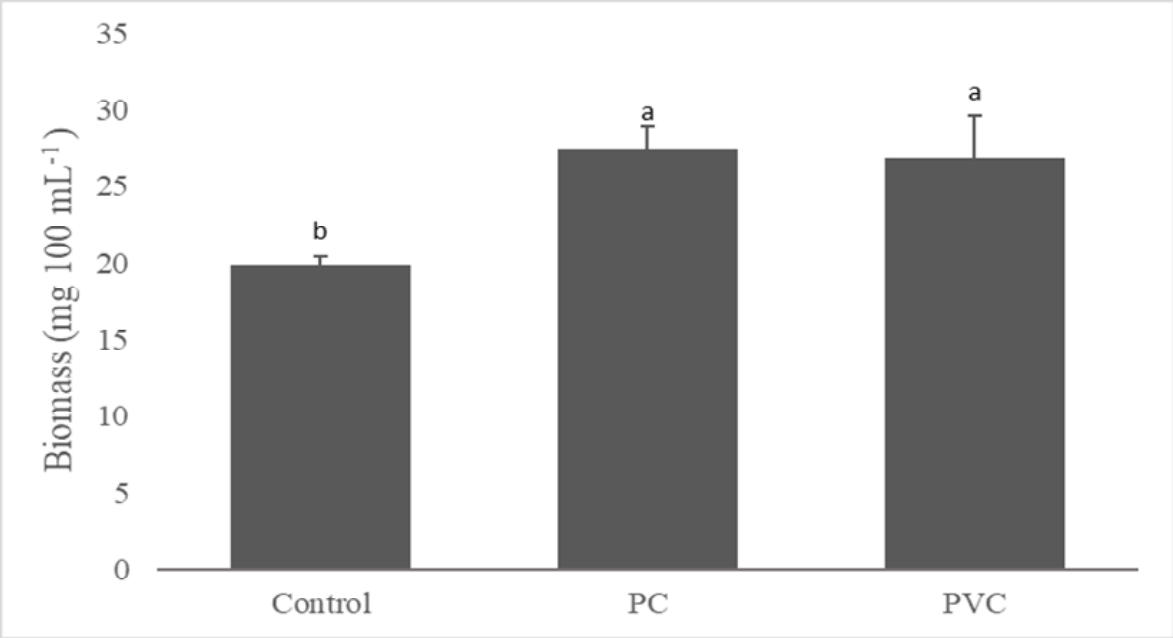

Figure 3. Dry weight biomass of *Thalassiosira* sp. under suspended and non-suspended cultivation methods. Values are means of three replicates ± SEM. Bars with different superscript letters indicate significant differences between treatments ($p < 0.05$).

Similar results were reported by Nodque *et al*. (2024), who observed an increase in biofilm biomass on polycarbonate under mixotrophic conditions. This suggests that the surface properties of polycarbonate may promote biofilm formation regardless of the cultivation mode. The use of plastic substrates also facilitates harvesting biomass more easily through scraping, as biofilms exhibit a consistency similar to biomass obtained by centrifugation. In addition to substrate properties, cell density plays a crucial role in determining the biomass productivity and lipid content of microalgae (Thoisen *et al*., 2020). In this study, *Thalassiosira* sp. exhibited greater adhesion to both substrates at peak growth, leading to increased biomass production compared to the control.

Other factors affecting microalgal biomass accumulation include cultivation conditions, light intensity, CO_2 concentration, aeration, and nutrient availability (Gatamaneni *et al*., 2018; Coşgun *et al*., 2021). The results of this study indicate that PC and PVC substrates provided favorable conditions for *Thalassiosira* sp. colonization, resulting in high biomass production. Furthermore, the attached cultivation method demonstrated greater efficiency than the

suspended method due to its higher biomass yield and ease of harvesting.

Effect of different substrates on microalgal lipid content

Figure 4 shows the lipid content of *Thalassiosira* sp. from different treatments. A significant difference ($p < 0.05$) in lipid content on a dry weight basis was observed among the treatments, with the PC substrate attaining the highest average lipid content (8.50%), followed by PVC (7.45%). The lipid content of *Thalassiosira* sp. cultured on PC and PVC substrates was significantly higher than the control by 167.30% and 134.28%, respectively.

These findings align with Gómez-Ramírez et al. (2019), who reported that the lipid content of *Navicula* sp. and *Navicula incerta* in plastic substrates ranged from 5.38 to 8.72%. Similarly, Shen *et al.* (2015) found that the lipid contents in *Botryococcus braunii* biofilm were higher than in suspended cultures. Additionally, Nodque *et al.* (2024) reported the highest lipid content of 9.89% in *Thalassiosira* sp. under mixotrophic-biofilm culture, while this study observed a lipid content of 8.50% on polycarbonate under autotrophic conditions, with a slight difference of 1.39%. The observed difference might be attributed to the variations in nutrient availability between the two trophic conditions. The addition of organic carbon sources, such as glycerol, in mixotrophic culture can enhance lipid production compared to autotrophic conditions, where carbon dioxide is the primary carbon source. Despite these differences, the results of both studies are comparable, suggesting that biofilm formation plays a role in lipid production, regardless of the cultivation mode.

The results of this study indicate that using PC and PVC substrates for cultivating *Thalassiosira* sp. can enhance lipid accumulation. Given its high lipid content under attached cultivation conditions, *Thalassiosira* sp. could serve as a promising alternative lipid source, reducing reliance on wild-caught fish. Bhattacharjya *et al.* (2020) further reported that *Thalassiosira* sp. exhibited the highest EPA (25.54 mg g^{-1} dry cell weight) and DHA (25.238 mg g^{-1} dry cell weight) levels among the species investigated. In addition to EPA and DHA, *Thalassiosira* sp. is also rich in linoleic acid, α-linolenic acid, and ã-linolenic acid.

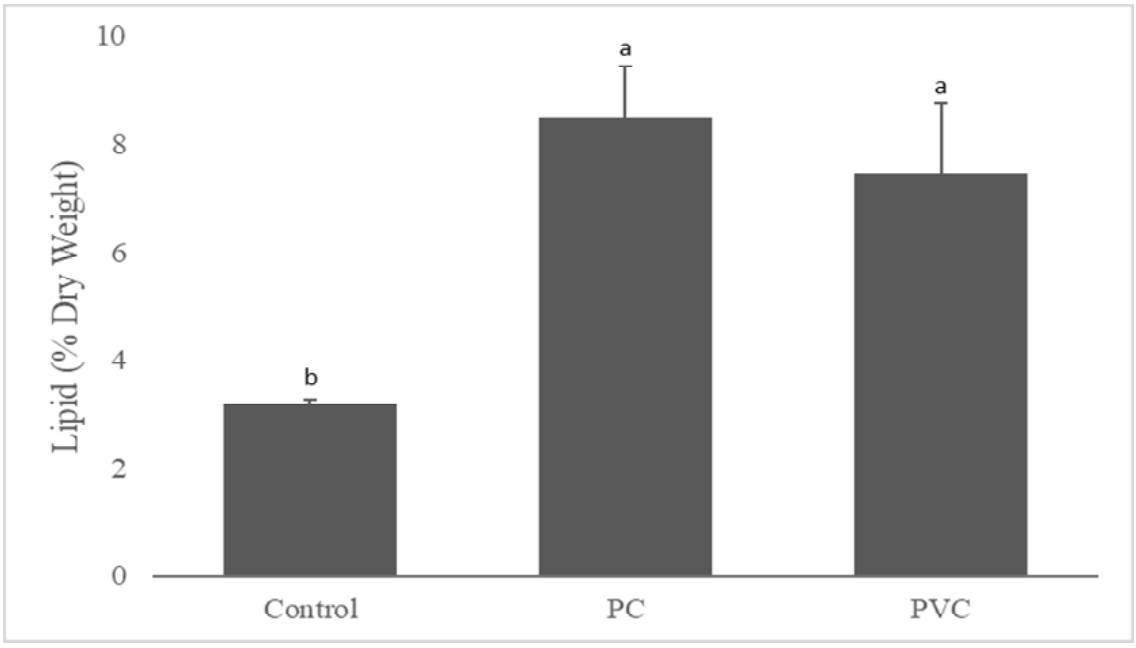

Figure 4. Lipid content of *Thalassiosira* sp. under suspended and non-suspended cultivation methods. Values are means of three replicates ± SEM. Bars with different superscript letters indicate significant differences between treatments *($p < 0.05$)*.

CONCLUSIONS

In summary, this study demonstrated that the attached cultivation method significantly improved the growth performance, biomass yield, and lipid content of *Thalassiosira* sp. compared to the suspended process. Both PC and PVC were effective as supporting materials for *Thalassiosira* sp. cultivation, promoting higher algal adherence and biofilm formation.

Further study is recommended to explore the effects of specific PC and PVC substrate properties, such as surface roughness and hydrophobicity, on the growth, biomass yield, and lipid content of

Thalassiosira sp. This information could aid in the development of optimized PC and PVC surfaces to boost microalgal productivity. Additionally, future studies should investigate lipid composition to assess the potential of *Thalassiosira* sp. as a sustainable lipid source for aquaculture feed, biofuels, and other bio-based products.

Overall, this study paves the way for further optimization of diatom-biofilm based cultivation, positioning *Thalassiosira* sp. as a promising candidate for various biotechnological applications.

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