

RESEARCH ARTICLE

Effects of Phosphate Nutrients on the Growth of the Marine Microalga *Navicula* sp. Isolated from the Eastern Coast of Thailand

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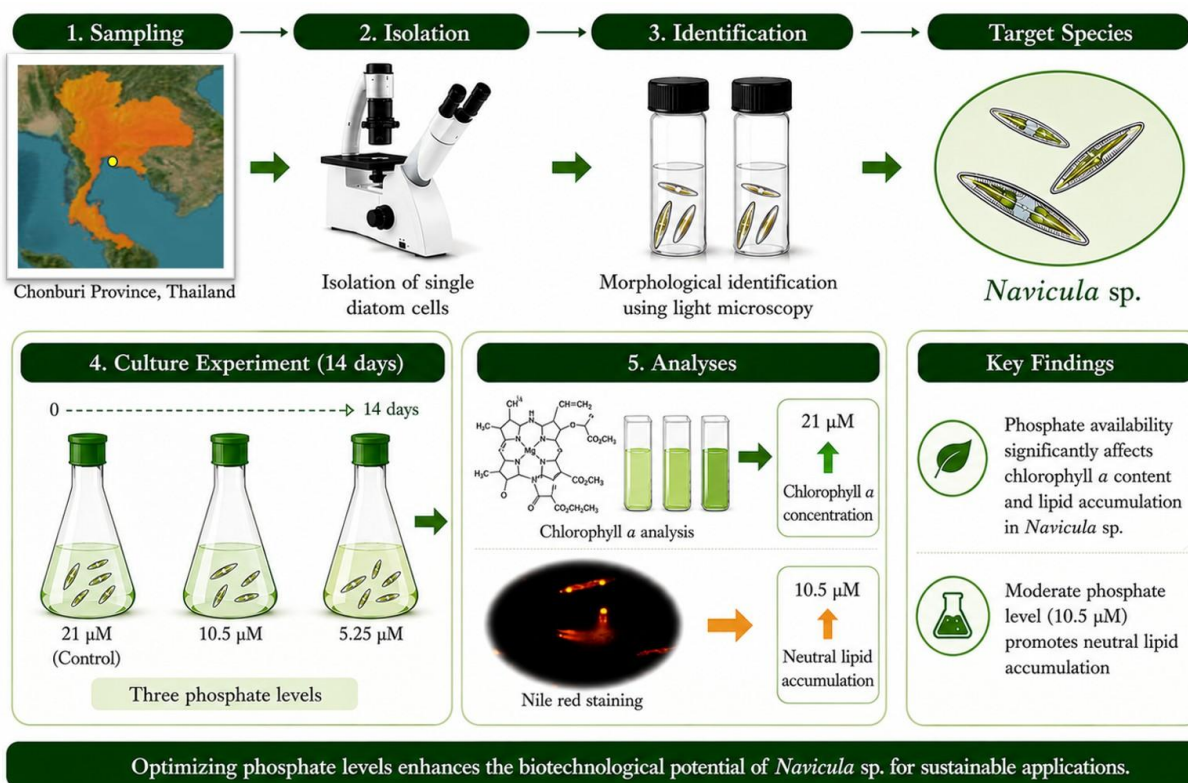
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GRAPHICAL ABSTRACT



ABSTRACT

Navicula sp., a marine diatom, plays a crucial role in aquatic ecosystems and holds significant potential for applications in aquaculture and biotechnology. This study aimed to investigate the effects of varying phosphate concentrations on the growth and photosynthetic efficiency of *Navicula* sp. TISTR 11108, isolated from the eastern coast of Thailand. Cultures were maintained in F/2 medium with three phosphate levels: 21 μM (control), 10.5 μM , and 5.25 μM . Growth was monitored over 14 days by measuring cell density and chlorophyll *a* (Chl *a*) content. The control group exhibited the highest growth performance, with a peak cell density of approximately $9.4 \pm 0.35 \times 10^5$ cells/mL, a Chl *a* concentration of 16.68 ± 0.79 mg/mL, and a specific growth rate of 0.28 ± 0.02 per day, indicating optimal photosynthetic activity. In contrast, cultures subjected to lower phosphate concentrations showed reduced growth and Chl *a* accumulation, accompanied by earlier signs of cellular degradation. Nile Red staining revealed lipid droplet formation in all treatments; however, the 10.5 μM phosphate treatment resulted in the highest lipid accumulation, followed by the 5.25 μM and 21 μM treatments. These findings highlight phosphate as a critical nutrient influencing diatom growth, while demonstrating that moderate phosphate limitation (10.5 μM) uniquely enhances lipid accumulation in *Navicula* sp. TISTR 11108. Overall, the results indicate a dual role of phosphate in regulating both photosynthetic efficiency and metabolic allocation, with important implications for biomass productivity, biofuel applications, and biotechnology.

Keywords: Growth, Phosphate, Marine microalgae, *Navicula*, Diatom

1. INTRODUCTION

Marine microalgae, particularly diatoms of the genus *Navicula*, are integral to aquatic ecosystems and hold substantial promises for biotechnological and aquacultural applications. As benthic primary producers, *Navicula* species contribute significantly to global carbon fixation through photosynthesis, thereby supporting marine biodiversity and regulating oceanic carbon cycles. Their capacity to form biofilms enhances sediment stability and facilitates nutrient recycling, promoting ecological balance in coastal habitats (Beaugrand *et al.*, 2010). Moreover, *Navicula* exhibits high lipid productivity and biosorption capabilities, making it a valuable candidate for biofuel development and wastewater treatment (Fernández *et al.*, 2025). In aquaculture, *Navicula* serves as a nutrient-rich feed for economically important species such as abalone (Kim *et al.*, 2019), fish and shrimp (Wardi *et al.*, 2023), thereby improving growth performance and sustainability in seafood production (Daume *et al.*, 2003).

Nutrient dynamics, particularly the availability of macronutrients like phosphorus, are critical determinants of micro-algal growth, species composition, and metabolic output. Phosphorus, primarily assimilated in the form of phosphate (PO_4^{3-}), is a vital element involved in nucleic acid synthesis, energy metabolism via adenosine triphosphate (ATP), and membrane formation through phospholipids. It also plays a pivotal role in enzymatic phosphorylation, which drives photosynthetic carbon fixation and cellular respiration. Phosphate limitation can severely impair microalgal cell division, photosynthetic efficiency, and biomass accumulation, while excessive phosphate levels may trigger eutrophication and harmful algal blooms (HABs), disrupting aquatic ecosystem stability (Yang *et al.*, 2014; Amalah *et al.*, 2018).

Microalgae have evolved adaptive mechanisms to cope with fluctuating phosphorus availability, including the activation of high-affinity phosphate transporters and the synthesis of intracellular phosphorus storage compounds such as polyphosphates. These strategies not only enhance survival under nutrient stress but also influence physiological traits such as lipid accumulation and growth rate, traits that are crucial for biofuel production and aquaculture feed optimization. Notably, species-specific responses to phosphate availability have been observed; for instance, *Chaetoceros muelleri* demonstrates increased lipid accumulation under phosphorus limitation (Lovio-Fragoso *et al.*, 2019), whereas *Chlorella vulgaris* shows enhanced biomass production under phosphorus enrichment (Tavares *et al.*, 2023). Given the ecological significance and biotechnological potential of *Navicula*, understanding the effects of phosphate nutrients on its growth is essential for optimizing its use in aquaculture systems. This study aims to evaluate the influence of phosphate concentrations on the growth dynamics of *Navicula* sp. TISTR 11108, which is isolated from the eastern coast of Thailand, thereby contributing to the development of sustainable microalgal cultivation strategies for aquaculture, biofuel production, and biotechnology.

2. MATERIALS AND METHODS

2.1 Collection of microalgae

The microalgal sample was collected from the bank of Suan Sukaphab Koh Loi, Sriracha, Chonburi Province (13°10'21.8" N 100°55'30.2" E). The collected sample was put in a plastic bottle containing artificial seawater of sterilized F/2 media (Guillard and Ryther 1962; Berges *et al.* 2001) to keep microalgae alive and to allow them to grow, and then transported to the laboratory for identification.

2.2 Separation of microalgae

Microalgae were separated using the micropipette washing technique. Briefly, the glass Pasteur pipettes were sterilized at 121°C for 15 minutes and placed in an oven at 50–60°C until dry. The tip of a glass Pasteur pipette was heated in a flame until soft and then stretched until getting tiny tube. A single cell from the collected sample was picked up under a microscope and released cell in a tube containing the artificial seawater of F/2 sterilized media (Guillard and Ryther, 1962; Berges *et al.*, 2001). Tubes were placed under fluorescent light at an intensity of 10-20 $\mu\text{mol photons/m}^2/\text{s}$ for a duration of 1–2 weeks. After this period, examine the cultures under a microscope to check for cell purity. Only a single algal species should be visible. If the culture is not yet pure, continue isolating the cells until a pure algal culture is obtained.

2.3 Preparation of the inoculum and media

Navicula cells were inoculated into 250 mL Erlenmeyer flasks containing 100 mL of artificial seawater prepared with F/2 medium. The medium consisted of the following components: NaCl (21.19 g/L), Na₂SO₄ (3.55 g/L), KCl (0.599 g/L), NaHCO₃ (0.174 g/L), KBr (0.0863 g/L), H₃BO₃ (0.0230 g/L), NaF (0.0028 g/L), MgCl₂·6H₂O (9.592 g/L), CaCl₂·2H₂O (1.344 g/L), and SrCl₂·6H₂O (0.0218 g/L). Nutrient solutions were added as follows: NaNO₃ solution (1 mL/L of 46.67 g/L), NaH₂PO₄·H₂O solution (1 mL/L of 3.09 g/L), Na₂SiO₃·9H₂O solution (2 mL/L of 15 g/L), and Fe-EDTA solution (1 mL/L containing FeCl₃·6H₂O at 1.77 g/L and Na₂EDTA·2H₂O at 3.09 g/L). A trace metal stock solution (1 mL/L) was added, containing ZnSO₄·7H₂O (0.073 g/L), CoSO₄·7H₂O (0.016 g/L), MnSO₄·4H₂O (0.54 g/L), Na₂MoO₄·2H₂O (1.48×10^{-3} g/L), Na₂SeO₃ (1.73×10^{-4} g/L), NiCl₂·6H₂O (1.49×10^{-3} g/L), and Na₂EDTA·2H₂O

(2.44 g/L). Additionally, a vitamin stock solution (1 mL/L) was supplemented with thiamine-HCl (0.1 g/L), biotin (0.002 g/L), and vitamin B₁₂ (0.001 g/L). Cultures were maintained under continuous shaking at 100 rpm and illuminated light (Cool White POWER MAX LED T8, 18 Watt) at a light intensity of $50 \pm 10 \mu\text{mol photons/m}^2/\text{s}$. The temperature was controlled at $28 \pm 2^\circ\text{C}$. Cultivation was carried out for 7–10 days until sufficient biomass was obtained for experimental use.

2.4 Culture conditions

Navicula sp. TISTR 11108 was cultured in F/2 medium (Guillard and Ryther, 1962; Berges *et al.*, 2001) prepared with defined concentrations of macronutrients and trace elements. Phosphate was supplied at three levels (21 μM , 10.5 μM , and 5.25 μM), while all other nutrients were maintained at non-limiting concentrations. Nitrate was provided at 549 μM , silicate at 105 μM , and trace metals (Fe, Mn, Zn, Co, Cu, Mo) and vitamins (B₁₂, biotin, thiamine) were added according to the F/2 formulation. These concentrations were selected to ensure sufficient availability for diatom growth and to prevent limitation by nutrients other than phosphate. Nutrient composition was verified at the start of each experiment, and only phosphate concentration was varied among treatments. This design ensured that observed differences in growth, chlorophyll *a* content, and lipid accumulation were attributable solely to phosphate availability. The experimental procedures were conducted using clear Duran glass bottles with a capacity of 1L, and each treatment was performed in triplicate. Each bottle was filled with 0.7L of sterilized artificial seawater prepared with F/2 medium containing three different phosphate concentrations. The control treatment consisted of NaH₂PO₄·H₂O at 3.09 g/L, resulting in a final phosphate concentration of 21×10^{-6} M. The second and third treatments used NaH₂PO₄·H₂O at 1.545 g/L and 0.7725 g/L, corresponding to final concentrations of 10.5×10^{-6} M and 5.25×10^{-6} M, respectively. Each medium was carefully prepared and individually dispensed into the designated glass bottles. *Navicula* cells were incubated for 14 days at $28 \pm 1^\circ\text{C}$ under continuous illumination provided by cool white, fluorescent lamps (POWER MAX, LED T8, 18 Watt) at a light intensity of $50 \pm 10 \mu\text{mol photons/m}^2/\text{s}$, following a 24-hour light regime. The bottles were plugged with cotton and gently aerated using sterile air passed through a 0.2-micron filter for the duration of the experiment.

2.5 Growth analysis

Cell counts for each culture were monitored daily except on weekends over 14 days using a hemocytometer. The specific growth rate (μ) of the microalgae was calculated using the following equation:

$$\mu = \ln (N_2/N_1) / (t_2 - t_1)$$

where: μ is the specific growth rate (day^{-1})

N1 and N2 are the biomass concentrations at time points t1 and t2, respectively.

2.6 Chlorophyll a analysis

Algal samples (5 mL per sample) were collected from each treatment, with three biological replicates. The samples were centrifuged, and each pellet was washed with distilled water 2 times to remove salt. The pellet was weighed by an electronic analytical balance (ML204T/00, Mettler Toledo, Switzerland). The sample was mechanically disrupted using a homogenizer (IKA dispersion S18N-10G, Germany) at 8,000 rpm for 2-3 minutes in the presence of 3 mL methanol as the extraction solvent. The experiment was conducted in a beaker with ice to maintain the

temperature of the crude extract at 5 ± 2 °C under dim light conditions since pigment is photosensitive. The extraction process was repeated, and each supernatant was pooled. Chlorophyll *a* content was determined using a spectrophotometer (Hitachi, U-2900, Japan). The samples were placed in a glass cuvette and tested for the absorption spectrum at 652.4 and 665.2 nm. Methanol was used as a blank. Measurements were performed in triplicate. Based on the absorption spectra, chlorophyll *a* content was determined according to the method described by Lichtenthaler and Buschmann (2001). The content of chlorophyll *a* was calculated using the following equations:

$$\text{Chl } a \text{ (}\mu\text{g/mL)} = 16.72 \times A_{665.2} - 9.16 A_{652.4}$$

Where: Chl *a* concentration is expressed in mg/mL, which is obtained by dividing the value in $\mu\text{g/mL}$ by 1,000.

2.7 Nile red staining

To detect intracellular lipid accumulation in microalgal cells, 10–20 μL of cultured algal suspension was transferred into a microcentrifuge tube. A volume of 50 μL of 25% (v/v) dimethyl sulfoxide was added to enhance membrane permeability, followed by gentle mixing for approximately 1 minute. Subsequently, 10 μL of Nile red solution (0.01 g/mL in acetone) was added to the mixture and vortexed briefly to ensure uniform staining. An aliquot of 5–10 μL of the stained suspension was pipetted onto a clean glass slide and covered with a coverslip. The prepared slide was immediately examined under a fluorescence microscope (BX-53, Olympus Japan). Chlorophyll autofluorescence was observed in the red channel, while neutral lipids stained with Nile red emitted yellow to orange fluorescence.

2.8 Statistical analysis

The experiment was performed in triplicate ($n=3$), and the data shown here are the mean $\pm$ standard error mean (SEM). The statistical analysis was done using IBM SPSS software.

3. RESULTS AND DISCUSSION

3.1 Identification of microalgae

Navicula sp. TISTR 11108 is identified and referred from the book “Identifying marine phytoplankton” (Tomas, 1997) and “Marine phytoplankton” (Hoppenrate *et al.*, 2009). *Navicula* is a boat-shaped diatom with elongated valves about 17–24 micrometers, bilaterally symmetrical valves that range from lanceolate to elliptical (**Figure 1A**). Each valve features a central raphe system for motility, bordered by fine striae composed of lineate areolae. The central area may be expanded, and valve ends vary from rounded to capitate. Under a microscope, *Navicula* cells often display a shimmering or translucent golden-brown appearance, especially when actively photosynthesizing. However, the exact shade can vary depending on environmental conditions, nutrient availability, and growth phase (Zhao *et al.*, 2014).

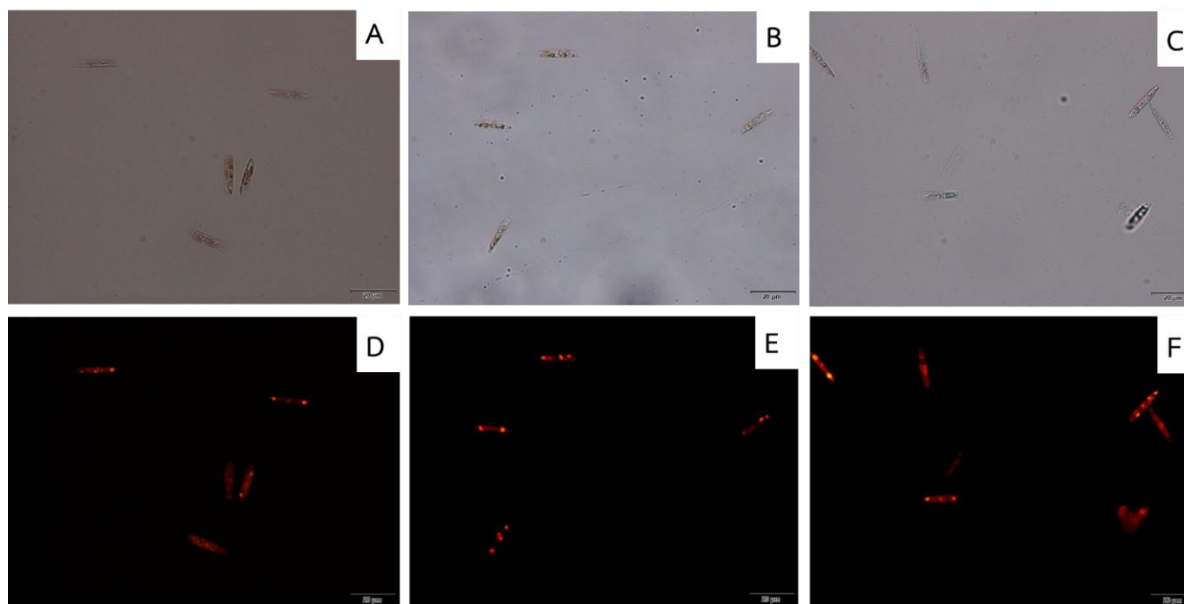


Figure 1 Microscopic morphological observation of *Navicula* sp TISTR 11108 growing under sterilized artificial seawater prepared with F/2 medium containing three different phosphate concentrations: 21 μM (A), 10.5 μM (B), and 5.25 μM (C) under the light micrograph and fluorescence micrograph of microalgae stained with Nile red of 21 μM (D), 10.5 μM (E), and 5.25 μM (F) at day 14. The yellow-orange fluorescence observed in the presence of Nile red indicates the presence of neutral lipids, whereas the red fluorescence corresponds to chlorophyll autofluorescence. Bars = 20 μm .

3.2 Growth of microalgae

The growth curve of *Navicula* sp. cultured in media containing three different phosphate concentrations ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$: 21 μM , 10.5 μM , and 5.25 μM) shows a distinct variation in cell content ($\times 10^5$ cells/mL) throughout a 14-day culture period. During the early culture phase (days 0–6), all treatments exhibited a gradual increase in cell density from approximately $3.3\text{--}3.6 \times 10^5$ cells/mL to about $5.9\text{--}9.4 \times 10^5$ cells/mL, indicating similar initial growth performance regardless of phosphate concentration (**Figure 2**). A rapid exponential growth phase was observed between days 6 and 8, where the cell density in the complete phosphate treatment (21 μM) reached the highest value of approximately $9.4 \pm 0.35 \times 10^5$ cells/mL and specific growth rate around $0.28 \pm 0.02 \text{ day}^{-1}$. In contrast, the half (10.5 μM) and quarter (5.25 μM) phosphate treatments reached lower peaks of around $8.5 \pm 0.50 \times 10^5$ and $7.0 \pm 0.43 \times 10^5$ cells/mL, and specific growth rate around 0.24 ± 0.02 and $0.18 \pm 0.02 \text{ day}^{-1}$, respectively. On day 8, statistical analysis revealed significant differences in cell density among treatments. Cultures exposed to 10.5 μM exhibited markedly higher cell density compared with both 21 μM and 5.25 μM treatments ($p < 0.05$). These findings indicate that the intermediate concentration (10.5 μM) provided the most favorable conditions for *Navicula* growth, while higher or lower concentrations were less supportive of cell proliferation. After day 8, all cultures showed a sharp decline in cell numbers, with the 21 μM phosphate treatment maintaining slightly higher cell densities during the stationary and declining phases compared to the phosphate-limited treatments. By day 14, the cell contents decreased to approximately $7.3\text{--}7.7 \times 10^5$ cells/mL across all conditions. The results clearly demonstrate that phosphate availability has a significant impact on the growth performance of *Navicula* sp.

Cultures supplied with complete phosphate (21 μM) achieved the highest biomass yield and sustained growth for a longer period, indicating that phosphate is a key limiting nutrient for diatom proliferation. The lower phosphate treatments (10.5 μM and 5.25 μM) showed reduced peak biomass and a more rapid decline phase, suggesting that phosphorus deficiency restricts cell division and accelerates nutrient depletion or stress-induced senescence. These findings are consistent with previous studies on diatoms and other microalgae. Li *et al.* (2016) reported that phosphate limitation led to a substantial decrease in cell density and chlorophyll *a* content in *Phaeodactylum tricornutum*, attributed to impaired energy metabolism and nucleic acid synthesis. Similarly, Reynolds (2006) noted that phosphorus deficiency often constrains diatom growth more severely than nitrogen limitation because of its central role in ATP and nucleic acid biosynthesis. The present results, therefore, align with established evidence that phosphate sufficiency enhances both growth rate and maximum cell yield in benthic and planktonic diatoms. However, while phosphate limitation reduces growth, it may also alter biochemical composition. Yao *et al.* (2019) reported that increased lipid accumulation in diatoms under phosphate stress. Thus, although biomass yield declines under low phosphate, such conditions might be favorable for targeted biochemical production, depending on the research or industrial objective.

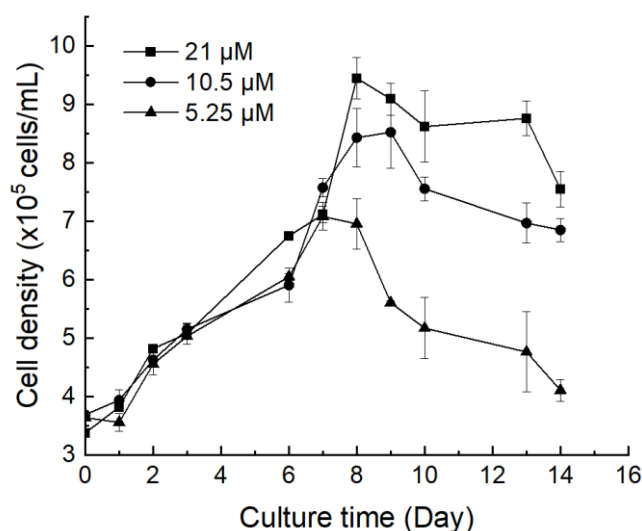


Figure 2 Growth curve of *Navicular* sp. TISTR 11108 cultured in three different phosphate concentrations ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$: 21 μM , 10.5 μM , and 5.25 μM) was observed for 14 days.

3.3 Chlorophyll *a* content

The chlorophyll *a* (Chl *a*) content of *Navicula* sp. cultured under different phosphate concentrations (21 μM , 10.5 μM , and 5.25 μM of artificial seawater F/2 medium) exhibited distinct growth-dependent variations over the 14-day cultivation period (**Figure 3**). During the early phase (day 0–6), Chl *a* content increased rapidly from approximately 4.19–4.40 mg/mL to 9.85–12.72 mg/mL across all treatments, indicating the onset of photosynthetic activity and cell proliferation. Cultures supplemented with 21 μM phosphate exhibited the highest Chl *a* content, reaching a maximum value of approximately 16.68 ± 0.79 mg/mL on day 8. This value was significantly higher ($p < 0.05$) than those observed in the 10.5 and 5.25 μM treatments at the same cultivation time. Although a gradual decrease in Chl *a* content was observed after day 8, values

in the 21 μM treatment remained significantly higher than in the lower phosphate treatments until day 14. In contrast, the culture with half phosphate supplementation (10.5 μM) attained a moderate peak of 10.82 ± 0.69 mg/mL at day 6, maintaining relatively stable levels (~ 8.38 – 9.43 mg/mL at day 8–14). The lowest phosphate concentration (5.25 μM) resulted in a significantly lower Chl *a* accumulation, peaking at around 9.85 ± 0.48 mg/mL on day 6 and subsequently decreasing to 5.01 ± 0.74 mg/mL by day 14.

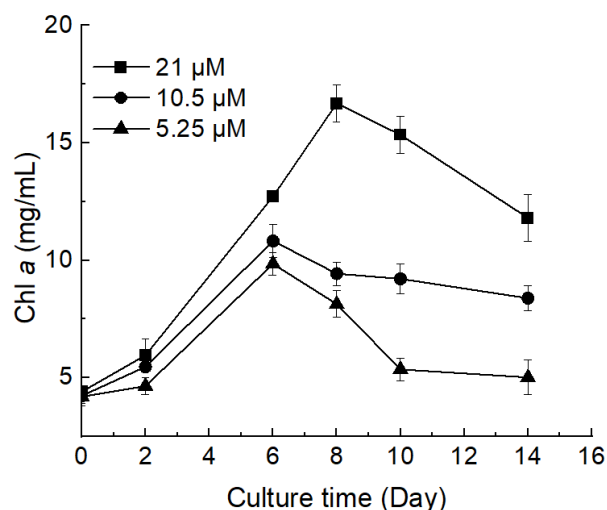


Figure 3 Chlorophyll *a* content (chl *a*; mg/mL) of *Navicula* sp. TISTR 11108 cultured in three different phosphate concentrations ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$: 21 μM , 10.5 μM , and 5.25 μM) observed for 14 days

These findings demonstrate a clear positive correlation between phosphate availability and photosynthetic pigment synthesis in *Navicula* sp. The higher phosphate level supported not only greater pigment accumulation but also sustained chlorophyll stability over time, indicating enhanced photosynthetic efficiency and metabolic activity under nutrient-replete conditions. Phosphorus is an essential macronutrient that plays a pivotal role in energy transfer, nucleic acid synthesis, and membrane structure in microalgae. The higher Chl *a* concentration observed under 21 μM phosphate treatment reflects improved photosynthetic performance and cellular energy metabolism, whereas phosphate-limited cultures (10.5 μM and 5.25 μM) exhibited reduced pigment accumulation and earlier pigment degradation. This reduction likely results from the impairment of ATP-dependent reactions in chloroplasts and restricted biosynthesis of photosynthetic proteins (Reynolds, 2006). Similar effects have been reported in other diatoms. Li *et al.* (2016) observed a significant decline in Chl *a* content and photosynthetic efficiency in *Phaeodactylum tricornutum* under phosphate-depleted conditions, correlating with lower energy metabolism and reduced cell division. Likewise, Baldia *et al.* (2007) found that phosphorus limitation in *Skeletonema costatum* led to decreased chlorophyll content and photochemical efficiency, suggesting that phosphate deficiency directly affects pigment stability and light-harvesting capacity. Although phosphate limitation suppresses chlorophyll synthesis and biomass productivity, it can induce physiological adaptations such as enhanced carbon storage or lipid accumulation. Yao *et al.* (2019) reported that *Phaeodactylum tricornutum* subjected to phosphorus stress redirected photosynthetic energy toward lipid biosynthesis, highlighting a

potential trade-off between growth and metabolite accumulation. Therefore, while phosphate sufficiency promotes pigment production and biomass yield in *Navicula* sp., phosphate-limited conditions might be exploited for targeted metabolic engineering or biofuel applications.

3.4 Fluorescent staining of neutral lipid using Nile red

Navicula cells observed under brightfield microscopy exhibit their natural morphology, including elongated, rod-like shapes and internal chloroplasts with greenish pigmentation (**Figure 1 A, B, C**). In contrast, the same cells were stained with Nile red and visualized under fluorescence microscopy. Nile red, a lipophilic fluorescent dye, selectively binds to neutral lipids within the cells, emitting yellow to orange fluorescence upon excitation. This enables precise localization of lipid bodies, which are otherwise invisible under brightfield conditions. Additionally, chlorophyll autofluorescence appears red, allowing simultaneous visualization of both chloroplasts and lipid droplets. Fluorescence approach significantly enhances the ability to assess lipid accumulation in microalgae, which is crucial for biofuel research and metabolic profiling (Ahamed *et al.*, 2022). This differential fluorescence allowed for clear discrimination between cellular compartments. Upon staining algal *Navicula* cells with Nile Red to visualize intracellular droplets, lipid accumulation was observed across all three treatment conditions at day 14. Algae cultured under a phosphate concentration of 10.5 μM exhibited the highest abundance of lipid droplets (**Figure 1E**), followed by those grown at 5.25 μM and 21 μM , respectively (**Figure 1D, F**). This ability to permeate intact algal cells and bind intracellular lipids without requiring solvent extraction makes it a valuable tool for rapid lipid screening. This technique significantly reduces sample preparation time and preserves cell integrity, making it suitable for high-throughput lipid quantification and screening of lipid-producing microalgal strains. Nile red staining is used widely in many algae such as *Chlorella vulgaris*, *Dunaliella primolecta* and *Chaetoceros calcitrans* (Govender *et al.*, 2012), *Scenedesmus* sp. (Ren *et al.*, 2013), *Tetraselmis suecica* (Halim and Webley, 2015), *Phaeodactylum tricornutum* (Katayama *et al.*, 2019), *Nannochloropsis* sp. (Ahamed *et al.*, 2022), *Chlamydomonas oblonga*, *Chlorella vulgaris*, *Chlorococcum oleofaciens*, *Scenedesmus acuminatus*, *Scenedesmus vacuolatus* (Reuscher *et al.*, 2025). Similarly, Lauritsen *et al.* (2024) reported that Nile red analogs are used for membrane packing analysis in living cells. Their work confirmed that Nile red's solvent sensitivity and lipid selectivity make it a powerful tool for subcellular imaging, especially in lipid-rich environments.

4. CONCLUSION

This study demonstrates that phosphate availability strongly influences the growth and photosynthetic efficiency of *Navicula* sp. TISTR 11108 is a diatom strain isolated from the eastern coast of Thailand with promising commercial potential. Cultures supplemented with 21 μM phosphate achieved the highest cell density, sustained growth, and maximal chlorophyll *a* accumulation, whereas phosphate-limited conditions (10.5 and 5.25 μM) resulted in reduced biomass and accelerated pigment degradation. Phosphate deficiency also triggered intracellular oil droplet formation, indicating a metabolic shift toward lipid biosynthesis. These findings highlight phosphate as a critical factor for optimizing biomass yield and pigment stability in microalgal cultivation, while also revealing a novel observation: moderate phosphate limitation (10.5 μM) enhances lipid accumulation in *Navicula* sp. TISTR 11108, despite reduced growth. This underscores phosphate's dual role—not only as a growth nutrient but also as a regulator of

metabolic allocation between photosynthesis and lipid storage. Given its native origin in Thailand and recognized bioresource value, *Navicula* sp. TISTR 11108 represents a viable candidate for applications in aquaculture, biofuel production, and environmental biotechnology. Future research should explore nutrient interactions and environmental parameters to support scalable and sustainable cultivation strategies.

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