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Research Paper

Shearing Stress-Induced Lipid Accumulation in *Chlorella* Cultivated in CO_2-Enriched Medium Modified with NaOH

Ragil Pandu Sadewo[1,2], Devi Bentia Effendi[1], Nur Faiizah Aqiilah Firman[1], Putu Yudha Ugrasena[3], I Gusti Ayu Nadia Prasta Unique[4], Gema Puspa Sari[1,5], Khairul Anam[1*]

[1]*Research Center for Applied Microbiology, Research Organization for Life Sciences and Environment, National Research and Innovation Agency (BRIN), Cibinong, Bogor, 16911, Indonesia*
[2]*Department of Biochemistry, Faculty of Veterinary Medicine, Udayana University, Denpasar, Bali, 80234, Indonesia*
[3]*Department of Pharmacology and Therapy, Faculty of Medicine, Udayana University, Denpasar, Bali, 80234, Indonesia*
[4]*Department of Pharmacology, Toxicology, and Pharmacy, Faculty of Veterinary Medicine, Udayana University, Denpasar, Bali, 80234, Indonesia*
[5]*Department of Biology, Faculty of Medicine, Syarif Hidayatullah State Islamic University, Jakarta, 15412, Indonesia*
*__Corresponding author__: khai004@brin.go.id

Abstract
Chlorella can produce MUFA and PUFA, such as linolenic acid, with an ideal ω-3: ω-6 ratio content (1:1). This study investigates the effects of centrifugation-induced shear stress on lipid metabolism and fatty acid composition in *Chlorella sorokiniana* and *Chlorella vulgaris* cultivated in CO_2-enriched TAP medium modified with NaOH as an inorganic carbon source. Shear stress was applied during the exponential growth phase to assess its influence on biomass productivity, biochemical composition, and lipid accumulation under nutrient-rich (P1) and nutrient-deficient (P2) conditions. Results revealed that *C. sorokiniana* showed superior adaptability to shear stress compared to *C. vulgaris*, showing a 64.7% increase in lipid content and maintaining higher unsaturated fatty acid (UFA) proportions, particularly polyunsaturated fatty acids (PUFAs). In contrast, nutrient deprivation in P2 promoted saturated fatty acid (SFA) accumulation as an energy storage adaptation. Biomass recovery in P1 indicated the need for nutrient availability in sustaining growth following mechanical treatments. The fatty acid profile of *C. sorokiniana* was dominated by UFA (67.2%), including ω-3 and ω-6 PUFAs, whereas *C. vulgaris* showed a higher SFA (42.2%). These findings suggest that moderate shear stress can stimulate lipid biosynthesis and improve the nutritional quality of microalgal lipids without compromising cell viability, provided sufficient nutrients are available. The combination of shear stress induction and optimized nutrient management offers a promising strategy to enhance microalgal lipid production for functional food and nutraceutical applications.

Keywords
Chlorella, Fatty Acid, Lipid Accumulation, Shear Stress

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1. INTRODUCTION

Fatty acids are the primary components of dietary fats derived from triglycerides and phospholipids, playing essential roles in human metabolism and health. Most naturally occurring fatty acids have even-numbered, unbranched carbon chains (C4-C28) and are classified based on the number of double bonds into saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and so forth (Chen and Liu, 2020; Saini et al., 2021). PUFAs, particularly omega-3 (ω-3) and omega-6 (ω-6), are essential bioactive compounds that must be obtained from dietary sources such as fish oil, chia seeds, soybeans, and sunflower (Kapoor et al., 2021; Saini et al., 2021). Overall, PUFAs play crucial roles in preventing and managing chronic diseases, including cancer, cardiovascular, autoimmune diseases, and hold significant potential for nutraceuticals and functional foods (Chen and Liu, 2020; Czumaj and Śledziński, 2020; Kapoor et al., 2021). However, due to rising demand and the limitation of conventional PUFA sources from fish and terrestrial plants, microalgae have emerged as promising alternatives.

Microalgae, such as *Chlorella sorokiniana* and *Chlorella vulgaris*, are promising nutritional sources due to their high content of proteins, lipids, essential fatty acids, vitamins, and other bioactive compounds. *Chlorella* can produce MUFA and PUFA, such as linolenic acid, with an ideal ω-3:ω-6 ratio content (1:1) (Mtaki et al., 2021; Papapanagiotou et al., 2024). These microalgae exhibit rapid growth, high efficiency, and superior

nutritional profiles, making them suitable for functional food applications (Mtaki et al., 2021; Vijay et al., 2025). Recognized as safe by the FDA, *Chlorella* is widely used in food products, though its rigid cell wall necessitates disruption to improve digestibility (Lorenzo et al., 2023; Vijay et al., 2025). Numerous studies support its potential to reduce blood pressure, cholesterol, and glucose levels, indicating relevance for the prevention of metabolic and cardiovascular diseases (Lorenzo et al., 2023; Papapanagiotou et al., 2024). Therefore, *Chlorella* is a promising candidate for nutritional supplements and health-promoting functional foods.

Chlorella is a unicellular green microalga *(Chlorophyta)* commonly cultivated using Tris-Acetate-Phosphate (TAP) medium with acetate as the primary carbon source. Under such conditions, glucose can be synthesized from acetate via the glyoxylate cycle without relying on photosynthesis (Mitra et al., 2021). In autotrophic cultivation, acetate can be replaced with sodium hydroxide (NaOH), an inorganic base that captures atmospheric carbon dioxide (CO_2) and converts it to bicarbonate (HCO_3^-), enhancing inorganic carbon availability for photosynthesis (Yu et al., 2021; Zhang et al., 2023). The combination of NaOH and CO_2 forms of sodium carbonate (Na_2CO_3) and bicarbonate ($NaHCO_3$), which improves CO_2 biofixation and biomass productivity (Padhi et al., 2025; Yu et al., 2021). Increasing inorganic carbon availability primarily supports efficient biomass generation, leading to the accumulation of metabolic precursors and stable autotrophic growth for subsequent metabolite induction strategies. This approach also promotes the accumulation of valuable metabolites, such as triacylglycerol (TAG), within microalgal cells.

Green microalgae are sensitive to several environmental stressors, including light intensity, temperature fluctuations, nutrient limitation, and hydrodynamic stress, such as shear stress. In a photobioreactor system, shear stress is commonly generated by aeration, mixing, and pumping processes, which create velocity gradients and turbulent flow within the culture medium (Pandur et al., 2020; Wang and Lan, 2018). These hydrodynamic forces influence cell morphology, membrane integrity, and metabolic activity and may stimulate lipid biosynthesis as part of cellular stress-adaptation mechanisms (Nguyen et al., 2024; Wang and Lan, 2018). Recent studies show that *Chlorella* cultivated in air-lift photobioreactors exhibits relatively high tolerance to aeration-induced shear stress and influences lipid synthesis, although excessive aeration increases shear rates to levels that pose a risk of cellular damage (Ding et al., 2021). In addition, algal granule formation and extracellular matrix composition are influenced by shear stress levels associated with agitation rates, with polysaccharide requirements increasing under low shear rate and protein requirements under high shear rate (Nguyen et al., 2024). Despite these advances, most previous studies have focused primarily on hydrodynamic shear stress generated by fluid flow within cultivation systems, while the potential effects of mechanical shear stress remain comparatively unexplored, indicating a significant gap in understanding how downstream processing steps may influence lipid metabolism.

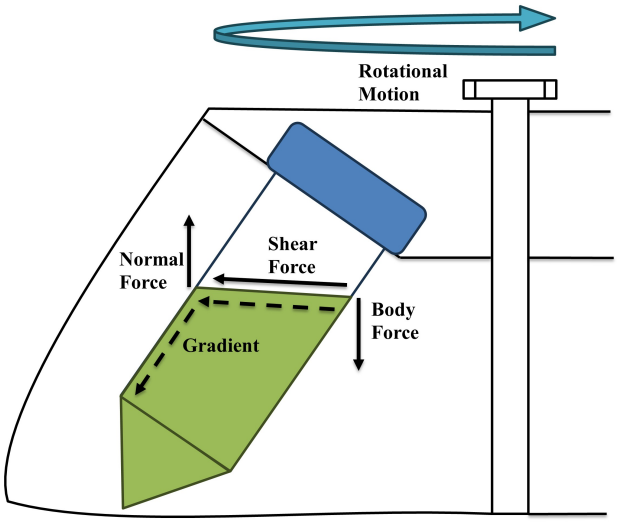

Figure 1. Shear Mapping During Centrifugation of *Chlorella* for Shear Stress Treatment

Various mechanical treatments, including high pressure, agitation, and centrifugation, can generate physical forces that interact with cellular structures and trigger mechanophysical responses. Among these, centrifugation produces transient mechanical forces characterized by high radial acceleration, rapid sedimentation, and localized velocity gradients, which can impose deformational stresses on microalgal cells (Dohan Ehrenfest et al., 2018; Nunes et al., 2024). This mechanical condition may induce distinct cellular responses, including changes in membrane tension, activation of mechanosensitive signaling pathways, and metabolic reprogramming associated with the lipid remodeling process (Nunes et al., 2024). These responses are potentially linked to increased lipid accumulation and PUFA biosynthesis (Yan et al., 2025; Yun et al., 2020). However, the role of centrifugation-induced shear stress in stimulating lipid accumulation and PUFA production in *Chlorella,* as illustrated in Figure 1, remains relatively underexplored and has not been systematically investigated.

Considering these aspects, understanding how mechanical shear stress affects microalgal physiology during cultivation remains essential for optimizing lipid productivity. Although numerous studies have extensively explored the influence of environmental and hydrodynamic factors on lipid accumulation, the potential role of mechanically induced stress during the cultivation phase remains poorly understood. In this context, centrifugation may act as a transient mechanical stimulus capable of altering cellular responses when microalgae are subsequently re-cultivated. Therefore, this study aimed to investigate the effect of centrifugation-induced shear stress on *Chlorella* lipid accumulation and fatty acid composition cultivated in CO_2-enriched medium modified with NaOH. In this sequential stage integration, the NaOH-modified medium supports autotrophic growth and biomass production through

enhanced inorganic carbon availability, while centrifugation-induced shear stress is applied as the primary trigger for lipid accumulation. By elucidating how shear stress applied during cultivation influences the metabolic responses, this research provides new insight into promoting lipid biosynthesis and improving the production of PUFAs in microalgae.

2. EXPERIMENTAL SECTION

2.1 Microalgae and Cultivation Conditions

The microalgae, *Chlorella sorokiniana* (LIPI12-Al016) and *C. vulgaris* (LIPI12-Al042), were obtained from the culture collection of the National Research and Innovation Agency (BRIN) (Bogor, Indonesia) and cultivated using Tris Acetate Phosphate (TAP) medium prepared according to the National Institute for Environmental Studies (NIES) formulation. Initial cultures were grown in 100 mL flasks containing 50 mL TAP at 30°C, 2000 lux continuous illumination, and orbital shaking (Anam and Sadewo, 2023; Sadewo et al., 2022). Cultures were initiated at OD_{750} = 0.1 (Forghani et al., 2022) and subcultured upon reaching OD_{750} = 1. Pre-cultures were prepared by inoculating 10% of the seed into fresh TAP and growing for 3 days before transfer to the main stock culture.

For maintenance, microalgae were cultured weekly in 250 mL Erlenmeyer flasks with 100 mL TAP medium at OD_{750} = 0.1 as working culture, with the third-week working culture replacing the stock culture after 1 month. pH optimization was conducted in 24-well microplates containing 1.5 mL TAP medium (pH 2–12), incubated at 30°C under 2000 lux continuous illumination (Sadewo et al., 2022). NaOH concentrations were initially screened in microplates, and selected conditions were scaled up in 1 L Scott bottles (Sadewo et al., 2022). The cultivation medium was adjusted to pH 6–7 by bubbling CO_2 into NaOH solution (1 L/min) before supplementation with TAP medium composition without a carbon source.

2.2 Experimental Design for Shearing Stress

In Stage 1, the microalgae were inoculated into 1000 mL bottles (Schoot, Germany) containing CO_2-Enriched cultivation medium modified with 60 mM NaOH and adjusted to a starting OD_{750} of 0.1 on day 0 (Okabe et al., 2026). Cultures were homogenized using a magnetic stirrer (35 × g) at 30 °C under continuous illumination (2000 lux) for 24 h (Bonnefond et al., 2017). On Day 2, microalgae cells were subjected to shear stress by centrifugation (3,500 × g, 5 min, 4°C) before being re-inoculated.

In Stage 2, the biomass was re-inoculated in the previous cultivation medium (P1) as a nutrient-rich medium and in phosphate buffer (0.1 M, pH 7) as a nutrient-deficient medium (P2). Untreated biomass was collected on Day 2 (H2) and maintained until Day 7 (H7) as initial and final controls. On Day 7, P1, P2, and H7 were harvested by centrifugation (6,000 × g, 5 min). These experiments were performed independently three times. The resulting biomass pellets were dried for 24 h Mohadi et al. (2017) and ground into fine powder.

2.3 Biochemical Responses to Shear Stress

Protein content by the Bradford method was assessed from 5 mg of biomass collected after shear stress treatment (Montone et al., 2018). Cells were disrupted with 0.5 mL of 1 M phosphate buffer and 0.5 mL of 0.2 mm glass beads using a bead beater at 900 × g for two cycles of 300 s. The homogenate was centrifuged at 3,500 × g for 2 min, and the resulting supernatant was used as the protein extract. Protein concentration was quantified using the Bradford assay (Merck), where 20 μL of extract was combined with 1 mL of Bradford reagent, vortexed for 10 s, and incubated for 5 min before measuring absorbance at 595 nm. Bovine serum albumin (BSA) served as the calibration standard.

Carbohydrate content in biomass subjected to shear stress was analysed by the DuBois phenol–sulfuric acid method (Kim et al., 2020). A total of 0.1 mg of biomass was mixed with 1 mL of distilled water, 1 mL of 5% phenol, and 5 mL of concentrated H_2SO_4, vortexed for 10 s, and incubated for 30 min. Absorbance was recorded at 490 nm, with glucose used as the calibration standard.

Lipid content was determined in shear-stressed biomass using the Bligh–Dyer method (Sierra et al., 2017). A total of 100 mg of biomass was extracted with 3 mL of chloroform–methanol (1:1, v/v) and sonicated at 40 kHz for 2 h. The first crude extract was separated, and the extracted biomass was re-extracted with an additional 3 mL of solvent, sonicated for another 2 h before maceration for 24 h, and separated as the second crude extract. An equal volume of water (1:1, v/v) was then added to the total crude extract, followed by vortexing for 30 s and centrifugation at 1,500 × g for 5 min. The lower chloroform phase was collected, evaporated at room temperature, and weighed gravimetrically to determine the lipid content.

2.4 Profiling of Fatty Acid Composition

Fatty acids in the dried biomass were directly trans esterified into fatty acid methyl esters (FAME) following the Lewis method (Quilodrán et al., 2020). For each assay, 20 mg of biomass powder was treated with 3 mL of a transesterification reagent composed of chloroform, hydrochloric acid, and methanol (10:1:1, v/v) in screw-cap tubes. The mixture was vortexed for 10 s and incubated at 90°C for 120 min. After cooling to room temperature, 1 mL of distilled water was added and vortexed for 10 s, followed by three sequential extractions with 2 mL of hexane each, vortexed for 10 s per extraction. A 1 mL aliquot of the crude FAME extract was collected into 2 mL GC vials for fatty acid composition analysis using gas chromatography-mass spectrometry (GC–MS). This analysis enabled the precise characterization of the fatty acid profile in *C. sorokiniana* and *C. vulgaris*, providing insights into its potential as a rich source of nutritionally valuable and bioactive lipids for nutraceutical applications.

3. RESULTS AND DISCUSSION

3.1 Shearing Stress on Biomass Production

The dry weight of *C. sorokiniana* biomass in P1 decreased by 34.5% compared to H7 in, while P2 showed a more pronounced reduction of 148.1%, as shown in Figure 2 , with final weight of P1 and P2 of 481.1 mg/L and 223.3 mg/L, respectively (Table 1). However, compared to H2, the biomass in P1 increased by 112.4%, indicating a substantial recovery under nutrient availability, whereas P2 exhibited a slight reduction of 1.4%, reflecting the inhibitory effects of combined shear stress and nutrient deprivation. In comparison, *C. vulgaris* demonstrated a smaller reduction, as shown in Figure 2, with biomass decreased by 25.6% in P1 and 50.1% in P2 compared to H7, with final weights of 266.7 and 212.2 mg/L, respectively (Table 1). Compared to H2, *C. vulgaris* biomass increased by 20% in P1 but decreased by 4.5% in P2. These results suggest that *C. sorokiniana* showed more sensitivity to nutrient deprivation following mechanical treatments than *C. vulgaris* and highlight the role of nutrient availability in supporting biomass regeneration after centrifugation-induced stress.

The shear stress was intentionally applied during the exponential growth phase (day 2) to assess changes in cellular biochemical composition and to determine its impact on biomass accumulation. Early application during the transition to stationary phase was designed to maximize biomass productivity and capture stress-induced physiological responses. Centrifugation shows in Figure 1 offers a highly controllable and reproducible shear stress, making it a practical method for inducing environmental stressors. Centrifugal force creates hydrostatic pressure that can impose significant mechanical stress on microalgal cells. While fragile species such as *Dunaliella salina* may rupture at forces exceeding 5000 × g, *Chlorella* rigid cell walls can withstand such forces, although even lower levels (~3500 × g) are sufficient to induce cellular shear stress (Molina-Miras et al., 2019). These species have spherical morphology (5–10 μm diameter) and unilamellar cell wall (17–21 nm thickness) enriched with rigid glucosamine-based microfibrils, which provide enhanced structural integrity under shear stress (Ahmad et al., 2020). These findings suggest that shear stress with nutrient supply has a measurable but not detrimental effect on biomass production, while nutrient deprivation under stress leads to a significant decrease in biomass.

3.2 Shear Stress on Biochemical Proportion

Exposure to shear stress through centrifugation, as shown in Figure 1, significantly affected the biochemical composition of *Chlorella*, particularly affecting the relative proportions of proteins, carbohydrates, and lipids. To better interpret these changes, the biochemical profiles of stressed cultures were compared with two reference conditions, the initial biomass harvested on Day 2 and untreated biomass on Day 7. The day 2 condition represents the early growth stage, characterized by relatively low accumulation of storage metabolites, whereas day 7 reflects the later growth phase, in which carbohydrate content increased while lipid levels remained relatively stable. In contrast, cultures exposed to shear stress exhibited lipid accumulation accompanied by distinct carbohydrate responses.

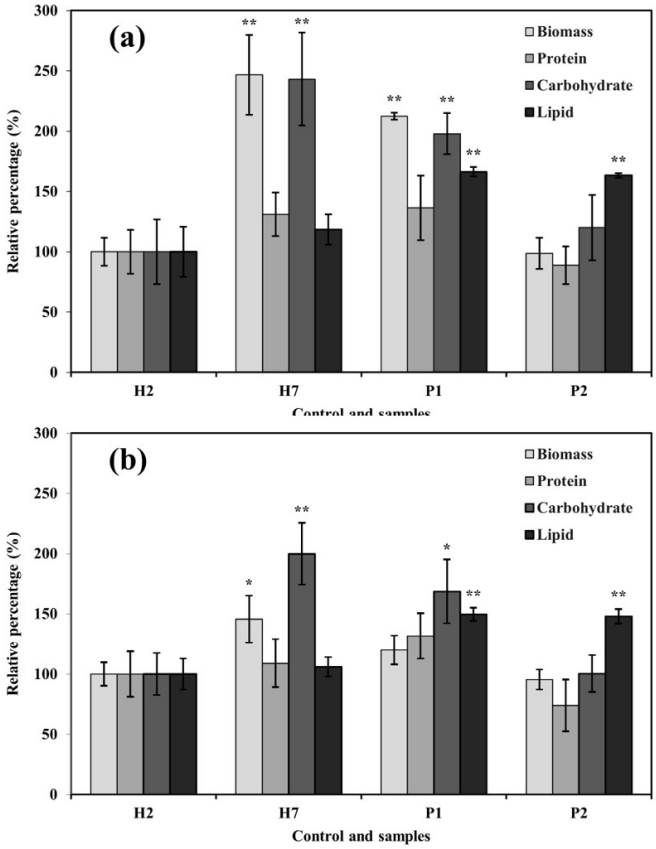

Figure 2. The Effect of Shearing Stress on *Chlorella* Metabolite pProduction. Metabolite Production of (a) *C. sorokiniana*, (b) *C. vulgaris*. The Values in Panels a and b are the Means and Standard Deviations of Three Independent Experiments. A t-Test was Used to Compare Treatments with H2 as the Control, with* = p-value <0.05 and ** = p-value <0.01

Total protein content during normal growth in both species is 22.3 and 16.2 mg/L in Table 1, showing variable responses depending on nutrient availability and mechanical stress exposures. In *C. sorokiniana*, the protein content in P1 increased by 36.5% compared to H2, whereas P2 showed a reduction of 11.2% (Figure 2). A similar pattern was observed in *C. vulgaris*, where protein levels increased by 31.6% in P1 relative to H2 but decreased by 26% in P2. Compared with H7, both species exhibited slightly higher protein levels in P1, with increases of 5.6% in *C. sorokiniana* and 23.2% in *C. vulgaris*, although these were not significant, suggesting that nutrient availability may buffer the physiological impact of shear stress. In contrast, protein content in P2 decreased markedly relative to H7, with reductions of 42.1% in *C. sorokiniana* and 34.7% in *C. vulgaris*. Shear stress, like vibration or centrifugation, has been shown to enhance the production of hydroxyproline-rich protein 3 (HRP3), an extracellular matrix protein associated

Table 1. The Metabolite Production of *Chlorella* Under Shearing Stress Treatment

Product	Concentration							
	Chlorella sorokiniana				*Chlorella vulgaris*			
	H2	H7	P1	P2	H2	H7	P1	P2
*Biomass production (mg/L)	226.5 ± 26.2^{A}	558.8 ± 74.9^{B}	481.1 ±6.3^{B}	223.3 ±29.2^{A}	222.2 ±21.6^{A}	323.5 ±43.2^{B}	266.7 ±26.5^{AB}	212.2 ±18.6^{A}
*Protein content (mg/g)	17 ±3.1^{A}	22.3 ±3.1^{B}	23.2 ±4.6^{B}	15.1 ±2.7^{A}	14.9 ±2.8^{A}	16.2 ±3^{B}	19.6 ±2.8^{AB}	11 ±3.2^{A}
*Carbohydrate content (mg/g)	207.3 ±55.4^{A}	504.2 ±79.9^{B}	410.3 ±35.6^{B}	248.8 ±56.3^{A}	258.3 ±45.2^{A}	516.2 ±66.2^{B}	435.3 ±68.4^{B}	259.4 ±39.7^{A}
*Lipid content (mg/g)	154.2 ±32.1^{A}	182.4 ±19.4^{A}	256.5 ±6.1^{B}	251.8 ±2.8^{B}	168.6 ±21.9^{A}	178.9 ±13.6^{A}	252.3 ±9.3^{B}	249.2 ±10.3^{B}
*Lipid Production (mg/L)	35 ±9.3^{A}	102.6 ±23.3^{B}	123.4 ±3.6^{B}	56.2 ±7.2^{A}	37.8 ±8.3^{A}	58.3 ±11.8^{AB}	67.2 ±6.3^{B}	52.8 ±2.8^{A}
*Fatty Acid content (mg/g)	100 ±17.3^{A}	120 ±30^{A}	200 ±17.3^{B}	190 ±17.3^{B}	100 ±17.3^{A}	130 ±17.3^{A}	200 ±30^{B}	190 ±17.3^{B}

*: Grouping was using the LSD method, α=0.05, n=3

with structural support (Paik et al., 2018). Thus, shear stress may contribute to improved protein synthesis, highlighting the potential for optimizing protein yields under controlled stress conditions.

Carbohydrate content showed distinct responses for different treatments in *Chlorella*. In *C. sorokiniana*, carbohydrate levels increased substantially between H2 and H7, indicating progressive accumulation of carbohydrate reserves during normal growth. However, under shear stress conditions, different responses were also observed. Carbohydrate content in P1 and P2 increased by 97.9% and 20.1%, respectively, compared to H2, but both treatments showed marked reductions relative to H7, which is 45.3% in P1 and 123.1% in P2 (Figure 2a). Similarly, carbohydrate content in *C. vulgaris* increased by 68.5% in P1 and only 0.4% in P2 compared to H2, while both treatments exhibited a reduction of 31.4% and 99.5%, respectively, compared to H7 (Figure 2b). These results suggest that while carbohydrates accumulate during normal cultivation, shear stress can alter carbon pathways. In nutrient-rich conditions, the carbohydrate levels remained relatively high despite lipid induction, whereas under nutrient-deficient conditions, carbohydrate reserves were markedly reduced. The redistribution of photosynthetically fixed carbon may enhance cell wall and membrane repair processes, restoring membrane stability and selective permeability within 2–6 days (Qu and Miao, 2021). This decline indicates that stored carbohydrates may be mobilized to support cellular maintenance and metabolic adaptation under stress conditions.

Lipid accumulation was substantially enhanced in both *C. sorokiniana* and *C. vulgaris* under shear stress. Lipid content in *C. sorokiniana* increased by 64.7% in P1 and 60.3% in P2 compared to H2, and by 45.2% in P1 and 40.8% in P2 relative to H7 (Figure 2a), confirming that shear stress is one of the inducers of lipid biosynthesis. Similarly, *C. vulgaris* showed lipid content increases of 52.3% in P1 and 48.8% in P2, respectively, from H2 of 40.2% and 36.7% compared to day 7 (Figure 2b). Despite similar cellular lipid content between the two stressed conditions, the total lipid yield per liter of culture medium varied significantly due to biomass differences shown in Table 1. Total lipid production of *C. sorokiniana* reached 123.4 mg/L in P1, a 59.5% increase from H7, while P2 yielded only 56.2 mg/L, representing a 132.5% decrease. In *C. vulgaris*, total lipid production increased by 23.9% in P1 and decreased by 14.5% in P2, with yields of 67.2 and 52.8 mg/g, respectively. *C. sorokiniana* showed better resilience and growth efficiency than *C. vulgaris*, particularly under room and elevated temperatures, upregulating lipid synthesis at the expense of carbohydrate pathways under many stress conditions (Li et al., 2018; Yun et al., 2020). These results suggest that while centrifugation-induced stress effectively stimulates lipid biosynthesis, nutrient availability remains essential to sustain biomass and optimize total lipid productivity.

At the metabolic level, lipid induction under shear stress can be linked to the redirection of carbon flux through central metabolic pathways. Photosynthetically fixed carbon in Figure 3 is first assimilated through the Calvin cycle and converted into glyceraldehyde-3-phosphate (G3P), which subsequently enters glycolysis to generate pyruvate, as reflected by the increase in carbohydrate between H2 and H7 (Cao et al., 2025; Zhang et al., 2023). Pyruvate is converted into acetyl-CoA, providing the key precursor for fatty acid biosynthesis through the fatty acid synthase (FAS) complex (Song et al., 2024). Under stress conditions, metabolic flux may shift toward acetyl-CoA production and G3P synthesis, facilitating the assembly of triacylglycerols via the Kennedy pathway. TAG accumulation represents a common adaptive strategy, enabling cells to store excess carbon while protecting cellular membranes in microalgae under various environmental stressors (Chen and Wang, 2021; Yang et al., 2022). This metabolic reallocation may explain why lipid accumulation increased in both P1 and P2.

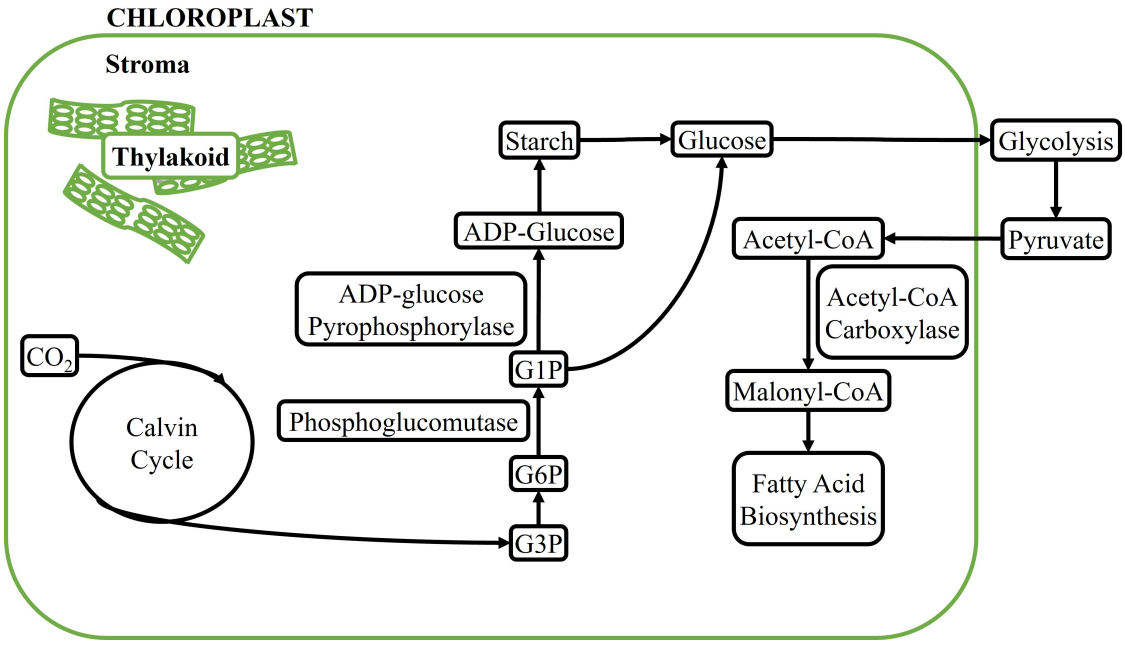

Figure 3. Carbon Fixation Pathway in *Chlorella* Cells Under Normal Growth Conditions

Although the lipid content per biomass increased under both shear-stressed conditions, total lipid production per liter of culture medium differed substantially due to variations in biomass productivity shown in Table 1. In *C. sorokiniana*, the total lipid production reached 123.4 mg/L in P1, representing a 59.5% increase when compared with H7, whereas P2 produced only 56.2 mg/L, corresponding to a 132.5% decrease due to reduced biomass growth. A similar pattern was observed in *C. vulgaris*, where total lipid production increased by 23.9% in P1 but decreased by 14.5% in P2, with yields of 67.2 and 52.8 mg/g, respectively. *C. sorokiniana* showed better resilience and growth efficiency than *C. vulgaris* under room and elevated temperatures, upregulating lipid synthesis at the expense of carbohydrate pathways under many stress conditions (Li et al., 2018; Yun et al., 2020). These results suggest that while centrifugation-induced stress effectively stimulates lipid biosynthesis at the cellular level, nutrient availability remains essential to sustain biomass and optimize the overall lipid yield per culture volume.

Overall, the comparison among H2, H7, P1, and P2 highlights distinct responses associated with growth phase and mechanical stress exposure. During normal cultivation, carbohydrates accumulated while lipid levels remained stable, reflecting typical carbon storage during the growth phase (H7). In contrast, shear stress treatments induced lipid accumulation under both nutrient conditions (P1 and P2). In P1, lipid accumulation occurred alongside high carbohydrate, suggesting that nutrient-rich conditions allow cells to maintain multiple carbon pools simultaneously. However, carbohydrate reserves declined substantially in P2, while lipid levels remained elevated, indicating a stronger redistribution of internal carbon toward lipid synthesis under nutrient limitation. These findings support the interpretation that centrifugation-induced shear stress acts as the primary trigger for lipid accumulation, whereas nutrient availability primarily influences biomass growth and the resulting lipid productivity per culture volume. Consequently, controlled application of shear stress under nutrient-sufficient conditions may represent a promising strategy for enhancing lipid productivity in microalgae.

3.3 Shear Stress on Fatty Acid Composition

The observed increase in lipid accumulation under shear stress also provides ad important basis for examining changes in fatty acid composition within the microalgal biomass. In microalgae, total lipid content alone does not fully determine the nutritional or biotechnological value of biomass, as the functional properties of lipids are influenced by their fatty acid profiles. Stress-induced lipid biosynthesis is often accompanied by modifications in fatty acid composition through modulation of fatty acid desaturation pathways that regulate the balance between SFAs and UFAs. The pronounced lipid induction observed in P1 and P2 raises the possibility that mechanical stress may also influence the distribution of fatty acids within the lipid fraction. To better understand the factual implications of shear stress-induced lipid accumulation, the fatty acid profiles under different treatments were further analyzed.

C. sorokiniana cultures in Table 1 showed 80% and 70% higher fatty acid content in P1 and P2, respectively, compared to H7, whereas *C. vulgaris* showed increases of 80% and 60% under the same conditions. *C. sorokiniana* and *C. vulgaris* contained 410.3 and 435.3 mg/g glucose in P1, respectively, and both cells produced approximately 200 mg/g fatty acids. Car-

bohydrate content dropped to 248.9 and 259.4 mg/g in P2, while both fatty acid content remained around 190 mg/g. This shift suggests a metabolic trade-off, in which reduced glucose production is accompanied by sustained fatty acid synthesis. Because glucose and fatty acids compete for carbon precursors derived from central metabolism, the decline in carbohydrate pools under shear stress reflects carbon flux redistribution toward fatty acid biosynthesis (Paik et al., 2018). Although fatty acid levels did not significantly differ between P1 and P2, the reduction in glucose indicates that carbohydrate metabolism may serve as an internal carbon source, consistent with previous findings that variations in lipid response are largely governed by microalgal tolerance to shear stress (Ding et al., 2021). These results indicate that shear stress acts as a metabolic driver, favoring lipid accumulation and positioning fatty acid biosynthesis as a key adaptive pathway under stress conditions.

Both *C. sorokiniana* and *C. vulgaris* followed a developmental trajectory pattern of fatty acid metabolism. PUFAs were predominant in H2, as shown in Table 2, supporting membrane biogenesis and maintaining fluidity during rapid cell division. As cultures progressed toward H7, fatty acid composition shifted toward increased MUFA, particularly oleic acid, which provides membrane stability and acts as an intermediate storage lipid. Such developmental changes are consistent with the regulation of fatty acid desaturase enzymes, including $\delta 9$, $\delta 12$, and $\delta 15$ desaturases, which introduce double bonds into fatty acid chains and control the conversion of SFAs into MUFA and PUFA (Murata et al., 1992). However, *C. sorokiniana* produced more lipid yield due to higher biomass productivity, resulting in a larger total fatty acid output than *C. vulgaris*. This baseline shift pattern highlights the dynamic regulation of fatty acid metabolism as an adaptive strategy to the growth stage.

Species-specific differences in fatty acid composition were also observed. UFAs accounted for approximately 67.2% of total fatty acids in *C. sorokiniana*, whereas SFAs represented 32.8% (Table 2). In contrast, *C. vulgaris* exhibited a more saturated profile with 57.8% UFAs and 42.2% SFAs. These differences indicate distinct regulatory strategies in lipid metabolism between the two species. This result indicates species-specific regulatory mechanisms in lipid metabolism. *C. sorokiniana* appears to maintain higher membrane fluidity through increased UFA synthesis, whereas *C. vulgaris* tends to allocate a greater proportion of carbon toward SFA as storage lipids during growth.

When shear stress was introduced on H2, the fatty acid profile of both *C. sorokiniana* and *C. vulgaris* in P1 diverged significantly from H7, as shown in Table 2. Instead of shifting into SFA accumulation, cells redirected carbon flux toward the synthesis of UFA, especially MUFA, reflecting an adaptive strategy as a protective function in preserving membrane flexibility and repairing shear-induced damage. The response in Figure 4 illustrates the dynamic regulation of lipid metabolism under stress, where UFA provides an advantage in sustaining cellular integrity (Barten et al., 2021). While shear stress is generally disruptive to membrane stability, the extended production of MUFA in P1 suggests that moderate mechanical treatments may act as a metabolic trigger to enhance the proportion of nutritionally valuable fatty acids in microalgal lipids.

The combined influence of shearing stress and nutrient deprivation in P2 revealed a more complex regulatory pattern. Although shear stress initially inhibits the shift from UFA to SFA, nutrient deprivation in P2 eventually forces the cells to prioritize SFA synthesis, particularly palmitic acid. This shift underscores the metabolic emphasis of energy conservation over membrane fluidity under unfavorable growth conditions. This adjustment highlights the function of SFA and MUFA as compact, oxidation-efficient storage molecules that provide greater long-term energy stability than carbohydrate reserves (Yaakob et al., 2021). Nevertheless, the cells in P1 retained higher proportions of UFA levels for a longer duration than H7, indicating that mechanical treatments can modulate membrane flexibility but cannot fully override the metabolic constraints imposed by nutrient deprivation.

Specific changes in fatty acid composition further illustrate these distinct adaptive responses between *C. sorokiniana* and *C. vulgaris* under shear stress. In *C. sorokiniana*, Vaccenic and oleic acids predominated in H7 during the MUFA-rich phase, whereas stearic acid was accumulated in P2, indicating the metabolic shift toward SFA accumulation. Importantly, ω-3 and ω-6 PUFAs such as linolenic acids declined steadily in H7 but were maintained at relatively higher levels in P1 and P2 during mid-growth, suggesting a protective metabolic adjustment. Considering that *C. sorokiniana* fatty acids production can accumulate for up to 74% of total biomass under room and elevated temperatures, including ω-3 and ω-6 PUFA (Remize et al., 2021; Yun et al., 2020). ω-3 PUFA are recognized for reducing cardiovascular disease (CVD) risks, improving vascular and neural functions, exerting anti-inflammatory and anticancer (Liu et al., 2022). In contrast, the synthesis of vaccenic or stearic acids in *C. vulgaris* was not as consistently observed in *C. sorokiniana*, and the proportions of PUFA remained largely unchanged. This indicates that shear stress not only influences the quantitative balance of fatty acids in *C. sorokiniana* but also enhances the qualitative profile, while *C. vulgaris* maintains the limited fatty acid composition for adaptive modulation.

The physiological and chemical implications of these interspecific findings are truly significant. The dominance of *C. sorokiniana* UFA production in P1, particularly PUFA, emphasizes the potential of shear stress induction as a strategy to enhance the production of bioactive fatty acids. Since PUFAs are highly valued for their health-promoting effects, combined with greater biomass yield, makes *C. sorokiniana* is a good candidate for functional food and nutraceutical applications, including potential applications in nanostructured lipid carriers for drug delivery (Zhihrotulwida et al., 2025). However, the eventual metabolic shift toward SFA in P2 highlights the need for integrated cultivation strategies that combine shear stress with optimized nutrient management to sustain desirable fatty acid profiles. Meanwhile, the metabolic stability of *C. vulgaris* could still be advantageous for applications requiring consistent lipid profiles, but its lower responsiveness limits the potential for

Table 2. The Fatty Acid Profile of *Chlorella* Under Shearing Stress Treatment

Fatty Acid	Concentration (%)							
	Chlorella sorokiniana				*Chlorella vulgaris*			
	H2	H7	P1	P2	H2	H7	P1	P2
SFA	29.38	32.85	28.07	35.54	34.26	42.25	38.19	42.35
UFA	70.62	67.15	71.93	64.46	65.74	57.75	61.81	57.65
MUFA	-	9.33	8.41	5.81	-	5.96	5.30	5.59
PUFA	70.62	57.83	63.52	58.65	65.74	51.80	56.51	52.06
C16:0	25.87	30.78	25.93	30.66	32.19	40.49	34.37	39.14
C16:2n6	15.17	12.06	11.77	7.58	9.15	7.04	7.79	3.20
C16:3n3	5.82	4.45	7.56	7.92	9.80	5.89	6.72	6.98
C18:0	-	-	-	1.94	-	-	-	-
C18:1n7	-	3.72	3.61	-	-	-	-	-
C18:1n9	-	5.02	4.16	5.32	-	5.71	4.77	5.17
C18:2n6	28.15	30.24	26.19	21.08	27.83	24.33	24.16	22.80
C18:3n6	13.03	7.43	13.15	17.22	15.00	12.38	12.19	15.14
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

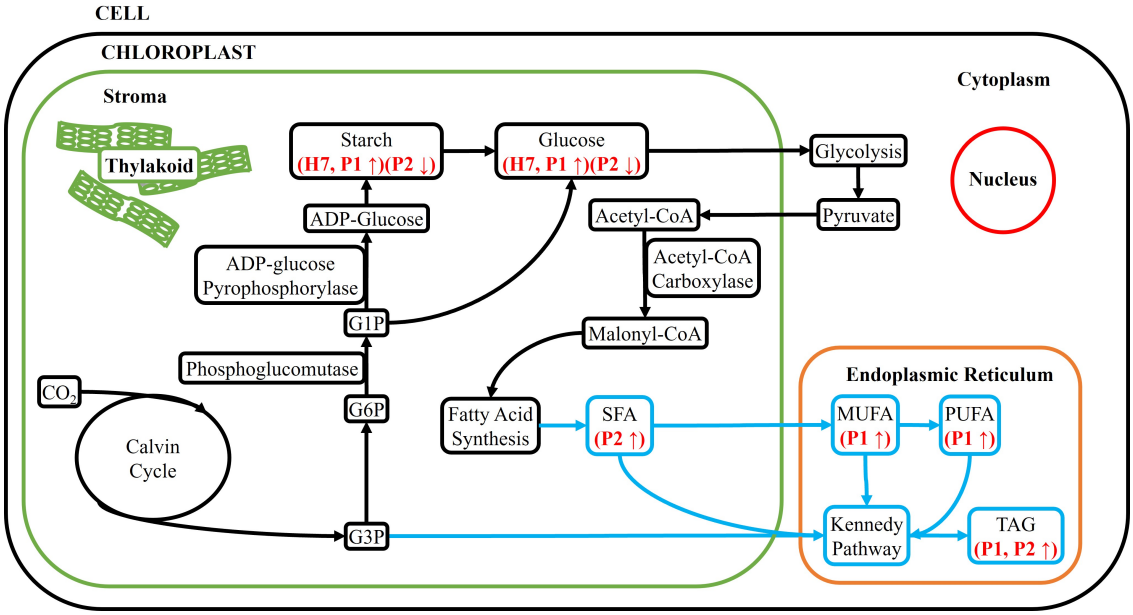

Figure 4. Mechanistic Model of Carbon Redistribution and Fatty Acid Biosynthesis in *Chlorella* Under Centrifugation-Induced Shear Stress, Cells Maintain Carbohydrate Pools While Increasing MUFA Production in P1, Whereas in P2, it Promotes Carbon Redistribution and SFA Accumulation

PUFA product development. Further analysis is needed to explain the details regarding the shift of PUFA to SFA, including oxidative stress markers and membrane integrity analysis.

4. CONCLUSIONS

This study demonstrates that centrifugation-induced shear stress significantly influences lipid metabolism and fatty acid composition in *C. sorokiniana* and *C. vulgaris* cultivated in modified medium. While both species exhibited enhanced lipid accumulation,*C. sorokiniana* showed superior adaptability, characterized by greater lipid productivity, higher UFA proportions, and more efficient metabolic remodeling. Nutrient availability was essential for sustaining biomass regeneration and optimizing lipid yield during shear stress, as nutrient-deficient conditions shifted metabolism toward SFA synthesis for energy storage. Future research should explore scalability in continuous systems and integrate advanced strategies such as dynamic mixing or microfluidic bioreactors to optimize productivity while maintaining cell viability. Additionally, combining biochemical profiling with omics approaches may provide deeper insights into the regulatory networks of fatty acid remodeling.

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