Physiological Characterization and Morphogenesis of an Edible Cyanobacterium, *Nostoc sphaeroides* Kützing, in Liquid Suspension Culture

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ABSTRACT

Nostoc sphaeroides Kützing, a cyanobacterium valued for its nutritional properties, is commonly used as both food and herbal medicine. Despite its significance, limited information exists on the morphological formation of its spherical colonies and the cultivation factors influencing their structure. This study investigates the formation of *N. sphaeroides* spherical colonies from single trichomes, providing detailed morphological and cellular characterizations. *N. sphaeroides* was identified by its scattered, straight trichomes, the formation of heterocytes and akinetes, and the development of spherical macrocolonies. Cultured in BG11 medium, *N. sphaeroides* showed significant morphological variation and biomass growth. The optimal growth conditions were observed at 50 rpm agitation, with incubation at 25±2 °C under a 16:8 light-dark cycle and a light intensity of 150 µmol photons $m^{-2} \cdot s^{-1}$. Under these conditions, the colony concentration, fresh weight, and dry weight reached their peaks on the 40th day, reaching 15.672 $g \cdot L^{-1}$ for fresh weight and 0.610 $g \cdot L^{-1}$ for dry weight. Key developmental stages were noted in the batch culture: aggregated cell masses by the 7th day, asteriated colonies between the 13th and 15th days, 300–350 µm microcolonies by the 21st day, and 9–13 mm spherical macrocolonies by the 30th day. As the colonies expanded beyond 13 mm after the 30th day, they differentiated into distinct layers, eventually forming discoid or flat colonies upon rupture. These findings provide valuable insights for the scale-up and field cultivation of *N. sphaeroides*.

Keywords: Biomass attainment, Cultivation, Cyanobacteria, Heterocytes, Macrocolonies, Spherical colonies, Trichomes

INTRODUCTION

Nostoc is a well-known genus of cyanobacteria commonly consumed as food in several Asian countries, including Japan, China and the Philippines, due to its abundant presence and culinary applications in specific regions. For instance, in the Philippines, *Nostoc commune* is primarily consumed in the northern part of Luzon (Briones-Nagata, 2007; Geronimo *et al*., 2023). In Japan and China, certain districts incorporate *Nostoc sphaeroides* into soups and salads (Briones-Nagata, 2007; Martinez-Goss *et al*., 2021). These examples highlight the diverse yet localized culinary applications of different *Nostoc* species across various cultural contexts.

Nostoc sphaeroides is a filamentous cyanobacterium with a wide range of physiological and biochemical properties, including a complex vegetative cell developmental cycle. Previous studies have indicated that *N. sphaeroides* possess numerous bioactive compounds with significant antimicrobial, antiviral, antitumor, and anticancer properties. Consequently, it has gained recognition as a valuable organism with substantial pharmaceutical and

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nutritional potential (Briones-Nagata, 2007; Deng *et al*., 2008a; Xu *et al*., 2019). The demand for high-quality *N. sphaeroides* has surged dramatically over the past decade. However, meeting this growing demand remains a challenge due to the limited and inconsistent supply of *N. sphaeroides*.

Traditional production methods, such as the collection of spherical colonies in rice paddies, have a long history but often result in unreliable yields with considerable variations in both quality and quantity, influenced by seasonal and climatic conditions (Deng *et al*., 2008a; Xu *et al*., 2019). For *Nostoc* species in general, different photobioreactor systems, including moving bed photobioreactors (MBPBR), aerosol-based photobioreactors, and thin-layer raceway ponds, have been tested for large-scale cultivation (Mouga *et al*., 2024). Efforts have also been made to culture *N. sphaeroides* on a large scale under both controlled indoor environments (e.g., 40 L glass tank systems and growth chambers) and outdoor systems (e.g., paddlewheel-driven raceway ponds) (Li and Gao, 2004; Deng *et al*., 2008b). However, indoor cultivation faces challenges such as low biomass yield, high production costs, and colony rupture. In outdoor systems, the highest productivity achieved was 6.58 g dry weight $m^{-2} \cdot d^{-1}$, with smaller colonies (1–2 mm) proving more effective as inoculum. Despite these efforts, relatively low productivity indicates the need for further research to optimize cultivation methods and improve yield in future large-scale production (Deng *et al*., 2008b). To ensure a consistent and reliable supply of *N. sphaeroides*, it is essential to develop efficient methods for mass cultivation under optimized conditions. However, achieving this goal remains challenging due to limited information of the critical factors governing colony formation and the absence of an effective photobioreactor design suitable for large-scale production. Addressing these challenges is crucial for establishing a dependable *N. sphaeroides* supply to meet the growing market demands. Previous studies have demonstrated that *N. sphaeroides* can be successfully cultured under controlled laboratory conditions. For instance, Deng *et al*. (2008a) documented that temperature and light intensity significantly impact the pigment composition of *N. sphaeroides*. Photosynthetic performance within a single macrocolony of *N. sphaeroides* varies across its gelatinous layers, with filaments from different layers exhibiting distinct pigment composition and photosynthetic rates. Additionally, the internal structure of *N. sphaeroides* varied distinctly under different growth temperatures. Colonies grown at temperatures above 30 °C are generally softer and more prone to disintegration compared to those grown at lower temperatures (less than 25 °C) (Martinez *et al*., 1985; Xu *et al*., 2019; Martinez-Goss *et al*., 2021). Recent study has reported that higher Ca^{2+} concentrations enhance spherical colony formation from vegetative filaments and restrict colony size expansion (Chen *et al*., 2021). This effect is likely due to Ca^{2+} binding to weak acidic groups in the polysaccharide sheath on the cell wall surface, which enhances microcolony formation and increases macrocolony rigidity. Adjusting Ca^{2+} concentrations could be a viable strategy to improve biomass productivity and control *N. sphaeroides* morphological features in artificial cultivation systems (Jiang *et al*., 2016; Xu *et al*., 2019; Chen *et al*., 2021).

Building on this foundation, we conducted a comprehensive research to monitor the morphogenesis of *N. sphaeroides*, from single trichomes to large spherical colonies, and characterize its cellular development in liquid suspension cultures. Additionally, we monitored biomass yield at varying shaking speeds to assess the potential of mass production of this important edible cyanobacterium.

MATERIALS AND METHODS

Culture and maintenance of Nostoc sphaeroides

N. sphaeroides BIOTECH 4089 was obtained from the Philippine National Collection of Microorganisms, BIOTECH-UPLB. The cultures were initially incubated at 25 °C under continuous fluorescent light for 16 h at an intensity of 150 µmol photons$\cdot m^{-2} \cdot s^{-1}$, followed by 8 h of darkness daily. Manual shaking of *N. sphaeroides* was performed 3–4 times per day. The cultures were maintained in BG11 growth medium, which was replenished weekly (every 7 days) (Arguelles, 2024).

Observation of growth and morphological cell types of Nostoc sphaeroides

To observe the growth and morphological cell types of *N. sphaeroides*, free trichomes (about 2 mL) were subcultured into 500 mL Erlenmeyer flasks containing 250 mL of BG11 medium. The cultures were incubated at 25 °C under continuous fluorescent light for 16 h at an intensity of 150 µmol photons·m^{-2}·s^{-1}, followed by 8 h of darkness daily. The experiment was conducted as batch cultures in BG 11 medium without replenishment during the culturing period (Diao and Yang, 2014; Arguelles, 2018; 2024). The differentiation of akinetes, heterocytes, vegetative cells, and hormogonia was examined using a microscope (CX31, Olympus, Japan) at 40× or 100× magnification. Over a 30-day incubation period, the distribution of different cell types was documented by counting cells from six randomly selected photomicrographs. For each sample, three distinct areas, each measuring 40×40 µm, were chosen for manual cell counting to ensure representative data of the overall culture population. This method enabled detailed tracking of the cell type dynamics over time, providing insights into the growth and development patterns of *Nostoc* filaments. The diameters of large spherical colonies were measured using a vernier caliper. In this study, microcolonies were defined as spherical colonies with a diameter of less than 0.3 mm, while macrocolonies were those exceeding 0.3 mm in diameters (Diao and Yang, 2014; Xu *et al*., 2019).

Cultivation of Nostoc sphaeroides at varying shaking conditions

Samples of *N. sphaeroides* were cultured in triplicate in 500 mL Erlenmeyer flasks containing 250 mL of BG11 medium. Cultures were incubated in a Laobao shaking incubator (Zhengzhou, China) at shaking speeds of 0, 25, 50, and 75 rpm. The initial absorbance of each culture was set to 0.1 at 750 nm, and incubation was conducted at 25±2 °C under a 16:8 light-dark cycle with a light intensity of 150 µmol photons·m^{-2}·s^{-1}.

Cyanobacterial growth was assessed by measuring the average biomass concentration of *N. sphaeroides* following the method by Diao and Yang (2014), with few modifications. Samples (1 mL) were aseptically collected from each culture treatment, and optical density (OD) was measured at 750 nm using a UV/Vis spectrometer (Hitachi U-2001), with BG 11 medium as the reference (Diao and Yang, 2014; Arguelles and Sapin, 2021). Growth was monitored every five days for 40 days by recording absorbance values to estimate biomass accumulation. At the end of the 40-day cultivation period, fresh and dry biomass weight of *N. sphaeroides* were determined. Dry cell weight was determined by drying harvested samples in an oven (FANEM Model 315 SE) at 80 °C for 24 h until a constant weight was obtained. Biomass measurements were performed using an analytical balance (model TE214S) with a resolution of 0.0001 g.

Cultivation of Nostoc sphaeroides at 50 rpm (optimum) shaking conditions

To further investigate biomass growth in BG11 medium, *N. sphaeroides* was subcultured into fresh BG11 medium and cultivated at 50 rpm (optimal shaking speed) for eight weeks. Cultures were grown in triplicates in 500 mL Erlenmeyer flasks containing 250 mL of BG11. The growth medium was replaced weekly with sterile, freshly prepared medium, and additional medium was added as needed to maintain a constant volume of 250 mL. Biomass was measured weekly.

Statistical analyses

Mean values and standard deviations were calculated for absorbance, dry weight, and fresh weight of *N. sphaeroides* grown under different shaking speeds. Each experimental setup was conducted in triplicate. Statistical significance among treatments was determined using analysis of variance (ANOVA), followed by Tukey's post-hoc pairwise comparison test. Statistical analyses were performed using Statistica v13, with significance set at $p<0.05$ (Arguelles, 2021).

RESULTS AND DISCUSSION

Life cycle of Nostoc sphaeroides

The mass propagation of *N. sphaeroides* requires a comprehensive understanding of its developmental stages, from initial trichome formation to the final development of microcolonies. This study monitored and analyzed each phase of growth, identifying key morphological changes and the environmental conditions that facilitate optimal growth and colony expansion.

Within four days, the filaments developed a gelatinous sheath (Figure 1a–1b). After approximately six days of incubation, hormogonia were observed (Figure 1b). The cell size of *N. sphaeroides* hormogonia typically ranged from 1–2 µm in diameter at their smallest to 3–4 µm at their largest. Following the appearance of hormogonia, heterocytes were detected after eight days of incubation. By the 11th day, young akinetes began differentiating among the elongated vegetative cells. Once the new akinetes matured, they re-germinated on the 13th day, leading to the elongation of new vegetative cells.

As cell division continued within the mucilaginous sheath, masses of aggregated cells developed along the algal filaments (Figure 1c–1d). These masses formed serial colonies, eventually developing into spherical colonies, either separated

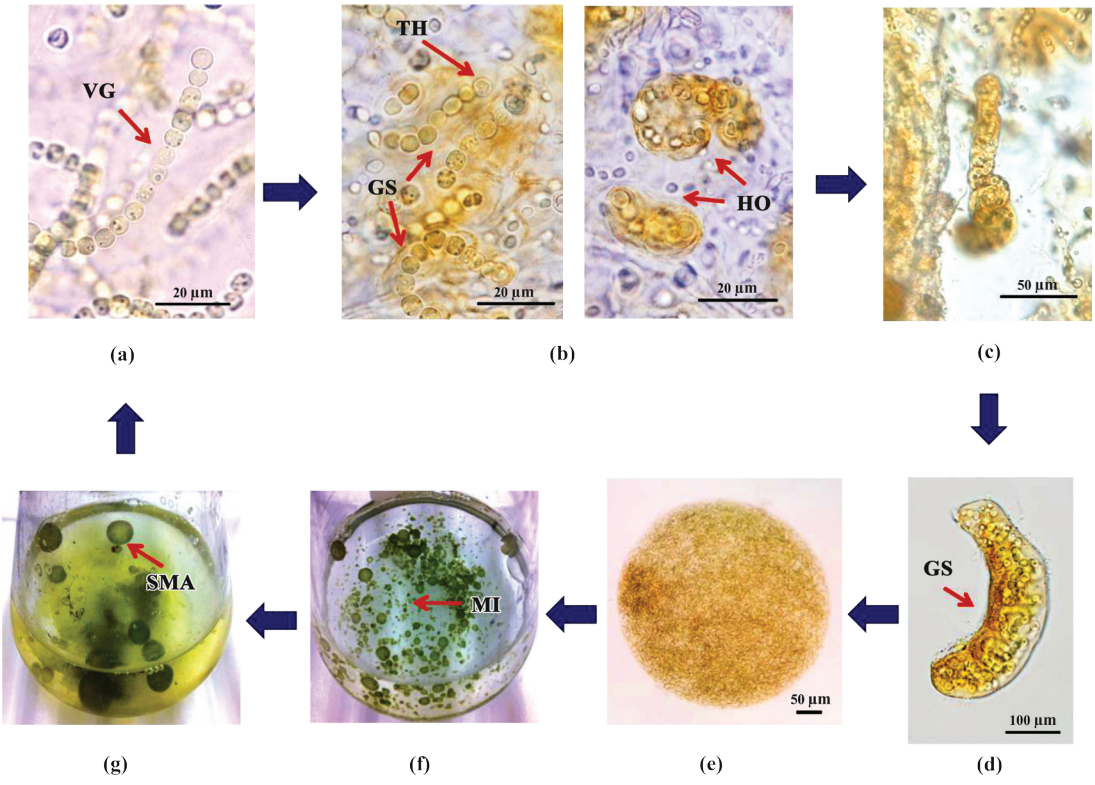

Figure 1. Cellular life cycle of *Nostoc sphaeroides* under batch culture. (a) Filaments showing co-existence of vegetative (VG) cells and an intercalary heterocyte (H); (b) Filament showing early formation of gelatinous sheath (GS) and terminal heterocyte (TH) as well as hormogonia (HO); (c) Aggregate of vegetative cells encapsulated with a gelatinous sheath; (d) Formation of short trichomes within a gelatinous sheath; (e) Young spherical colonies showing homogenous filaments; (f) Spherical microcolonies (MI) obtained after 21 days of cultivation; (g) Spherical macrocolonies (13 mm) (SMA) after 30 days of cultivation.

by heterocytes or remaining connected (Figure 1d) after 15 days. By the 21st day, microcolonies with diameters of 300–350 µm were observed (Figure 1e). Spherical macrocolonies, reaching diameters of 9–13 mm, were obtained by the 30th day (Figure 1g).

As microcolonies expanded into macrocolonies exceeding 13 mm in diameter, they differentiated into distinct layers. When macrocolonies ruptured, they formed discoid or flat shaped colonies. Colonies with diameters ranging from 0.1 to 0.5 mm, grown at room temperature under a light intensity of 150 µmol photons·m^{-2}·s^{-1}, consistently increased in weight and size (Figure 1f) as the growth medium was replenished weekly with fresh BG11 medium. The largest colony reached a diameter of 13 mm or more after 30 days of cultivation (Figure 1g).

Colony formation and rupture in Nostoc sphaeroides

Hormogonia or trichomes released from *N. sphaeroides* colonies of any size are capable of forming new colonies. Additionally, budding was observed as a means of multiplication in this cyanobacterium. Discoid or flat colonies typically formed when macrocolonies of *N. sphaeroides* ruptured. In this study, colony rupture occurred at various developmental stages, often attributed to nutrient deficiencies in the culture or suboptimal temperature conditions, which conforms the results observed by Deng *et al*. (2008a). The optimal growth conditions for *Nostoc* species in liquid suspension cultures generally include moderate light intensity (30–50 µmol photons·m^{-2}·s^{-1}), temperatures between 25–30 °C, and a pH of 7.5–8.0. Cultures should be agitated at 120–150 rpm to prevent self-shading and ensure even nutrient distribution, using a 12:12 or 16:8 light-dark cycle (Jiang *et al*., 2016; Xu *et al*., 2019; Chen *et al*., 2021). Nutrients such as nitrogen and phosphorus are essential, with BG-11 medium commonly used as a nutrient source. These conditions help maximize growth while minimizing stress, aligning with findings from various studies on *Nostoc* cultivation in liquid suspension systems (Chen *et al*., 2021).

However, despite these controlled conditions, colony rupture was still observed in previous studies, suggesting that additional factors may be involved. The mechanisms governing colony rupture remain unclear, and preventing this phenomenon poses a significant challenge for large-scale *N. sphaeroides* cultivation. Rupture may be linked to physical and physiological stresses within the colonies, such as internal pressure buildup due to cellular growth, gas vesicle formation, or other structural changes as the colony expands. These factors could compromise the integrity of the mucilaginous sheath that binds the cells together, leading to fragmentation. Additionally, fluctuations in osmotic pressure or mechanical agitation in liquid suspension cultures may exacerbate these stresses, further contributing to colony disintegration.

While colony rupture can release hormogonia or trichomes that seed new growth, it remains a major limitation for large-scale cultivation. Understanding the precise mechanisms underlying this process is crucial for optimizing biomass yield and maintaining the structural integrity of colonies during cultivation. Further research into the physical and environmental factors influencing rupture could help developing strategies to mitigate this issue, ensuring more stable and controlled growth conditions for *N. sphaeroides* in industrial applications.

Macrocolony development and cultivation of Nostoc sphaeroides

Previous research has extensively documented the life cycle of various edible *Nostoc* species primarily focusing on their microcolonies and filamentous structures (Deng *et al*., 2008a; Su *et al*., 2008; Chen *et al*., 2011; Diao and Yang, 2014). However, studies on macrocolony development have been limited, primarily due to a lack of knowledge regarding their cultivation under artificial conditions (Martinez *et al*., 1985; Deng *et al*., 2008a; Su *et al*., 2008; Diao and Yang, 2014). In this study, we successfully cultured macrocolonies of *N. sphaeroides*, achieving diameters of up to 13 mm for spherical macrocolonies and up to 17 mm for discoid colonies. This achievement enables further exploration of the morphological variation and biomass growth of *N. sphaeroides*.

Our findings provides a comprehensive overview of the developmental and morphological stages of *N. sphaeroides*, illustrating the transition from initial filamentous structures to mature discoid colonies. The research documented the progressive changes in morphology, from filaments to spherical colonies (microcolonies and microcolonies), and ultimately to discoid or flat colonies in later developmental stages. Initially, microcolonies formed from *N. sphaeroides* filaments through cell division, resulting in the development of aggregated cell masses along the algal filaments. After 21 days of cultivation, these microcolonies expand to diameters of 300–350 μm. Over time, they continued to grow, forming macrocolonies that reached diameters of 11–13 mm within 30 days. Beyond 50 days of cultivation, ruptured macrocolonies gave rise to discoid colonies.

By closely monitoring these morphological changes throughout the growth period, researchers can better estimate the developmental stages of *N. sphaeroides* colonies and refine techniques for artificial cultivation. This study highlights critical time points and morphological milestones, providing valuable insights for optimizing cultivation methods and improving the understanding of *Nostoc* growth dynamics.

Effect of cultivating Nostoc sphaeroides at varying shaking condition

The slow growth rate of edible *Nostoc* species has been recognized as one of the significant challenges in its artificial cultivation (Gao and Ye, 2003; Diao and Yang, 2014). In the current study, we demonstrated that extending the culture duration of *N. sphaeroides* leads to increased biomass concentration, both in fresh and dry weight. Notably, a shaking speed of 50 rpm proved to be particularly effective, resulting in faster growth compared to other tested speeds (Table 1 and Figure 2). At this speed, the colony concentration, measured by optical density at 750 nm, increased from 0.09 to 0.91. Across different shaking speeds, the maximum average colony concentration ranges from 0.51 to 0.91. This substantial growth rate suggests that our newly developed cultivation technique could be highly effective for the artificial propagation of *N. sphaeroides*, potentially overcoming previous obstacles associated with its slow growth.

Table 1 highlights the effect of cultivating *N. sphaeroides* for 40 days under varying shaking speeds. At 50 rpm, both fresh and dry biomass weights were significantly higher compared to cultures grown at 0, 25, and 75 rpm, suggesting that

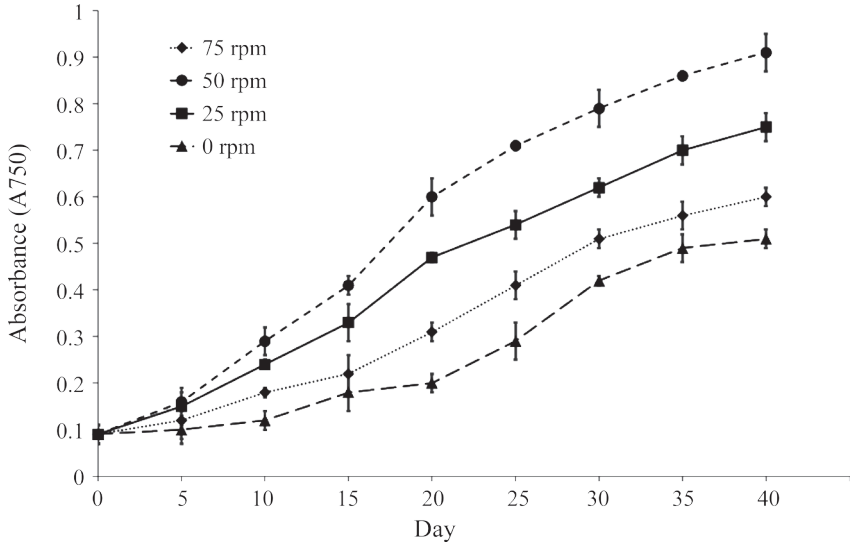

Figure 2. Growth trends of absorbance (A750) of *Nostoc sphaeroides* cultivated in liquid suspension under varying agitating speeds.

Note: Data points represent mean values with standard deviation bars.

Table 1. Specific growth rate, maximum fresh weight, and maximum dry weight yield of *Nostoc sphaeroides* grown in liquid suspension under varying shaking speed for 40 days.

Shaking Speed (rpm)	Specific Growth Rate (μ)	Maximum Fresh Weight ($g \cdot L^{-1}$)	Maximum Dry Weight ($g \cdot L^{-1}$)
0	0.163 ± 0.003^{a}	8.103 ± 0.16^{a}	0.132 ± 0.14^{a}
25	0.205 ± 0.002^{c}	13.198 ± 0.23^{c}	0.451 ± 0.22^{c}
50	0.241 ± 0.001^{d}	15.672 ± 0.19^{d}	0.610 ± 0.23^{d}
75	0.189 ± 0.002^{b}	11.472 ± 0.13^{b}	0.271 ± 0.19^{b}

Note: Mean±SD in each column, superscripted with different lowercase letters are significantly different ($p<0.05$) from each other ($n = 3$).

50 rpm is the optimal shaking speed for maximizing biomass yield. In stationary culture (0 rpm), *N. sphaeroides* colonies secrete exopolysaccharide-based substances that adhere to their surfaces, forming a protective layer known as an investment (Hill *et al*., 1994; Diao and Yang, 2014; Chen *et al*., 2021). This investment can hinder gas exchange, nutrient absorption, and light penetration, leading to light attenuation as it passes through the colonies. These factors likely contribute to the lower colony concentrations observed at *N. sphaeroides* cultures maintained under standing conditions (Su *et al*., 2008; Diao and Yang, 2014).

Conversely, at a shaking speed of 75 rpm, the increased physical agitation can cause colony rupture, preventing the formation of the exopolysaccharide investment, which is a stable structural component in *N. sphaeroides* and other cyanobacteria (Deng *et al*., 2008a; Su *et al*., 2008; Diao and Yang, 2014; Jiang *et al*., 2016). This rupture may occur in both macrocolonies and microcolonies during developmental growth due to the strong rotation force of the shaker and the renewal of BG11 medium. Rupture leads to colony disintegration and the release of filaments and hormogonia. At higher shaking speeds, microcolonies fail to develop into macrocolonies due to continuous bursting, and macrocolonies may fragment, forming new microcolonies that are unable to grow into stable discoid colonies (Kondratyeva and Kislova, 2001; Gao and Ye, 2003; Diao and Yang, 2014; Chen *et al*., 2021).

To optimize the mass production of *N. sphaeroides*, it is essential to control shaking speed to minimize colony rupture. Although the mechanistic process of colony bursting remains unclear, further study on its regulation could help facilitate the transition from microcolonies to macrocolonies and from macrocolonies to discoid flat colonies (Deng *et al*., 2008a; Diao and Yang, 2014; Nortez and Arguelles, 2023).

Shaking speeds significantly influences the growth and structural integrity of *N. sphaeroides* by influencing key factors such as light exposure, nutrient absorption, and colony stability. At an optimal speed, such as 50 rpm, uniform agitation enhances nutrient distribution, gas exchange, and prevents self-shading, leading to efficient nutrient uptake and improved light exposure. This results in increased biomass concentration and better colony development. In contrast, higher shaking speeds, (e.g., 75 rpm) induce mechanical stress, leading to colony rupture and preventing the formation of stable exopolysaccharide structures. Ruptured colonies exhibit reduced nutrient absorption and inefficient light utilization, ultimately limiting growth and hindering the development of microcolonies into macrocolonies. Thus, shaking speeds plays a critical role in regulating light exposure and nutrient absorption by altering the physical conditions within the culture. Lower speeds provide optimal growth conditions, whereas higher speeds induce stress, compromising colony integrity and overall biomass yield.

The fresh and dry weights of *N. sphaeroides* colonies grown at 50 rpm for 8 weeks were consistently proportional to their concentrations, indicating a clear correlation between colony

biomass and culture density. This proportionality highlights the efficiency of the selected shaking speed, as it not only promoted optimal growth but also mitigated issues such as self-shading and nutrient depletion, both of which can negatively impact biomass production. The results suggest that 50 rpm shaking speed could be effectively scaled up for mass cultivation of *N. sphaeroides* in industrial settings, where maximizing biomass yield is critical. By maintaining consistent agitation, light distribution, nutrient availability, and gas exchange within the culture are enhanced, leading to more uniform and robust growth (Figure 3). This finding underscores the potential of optimizing agitation speed as a key parameter for large-scale production of *N. sphaeroides* for applications such as biofertilizers, bioremediation, and biofuel production.

Cultivation parameters play a critical role in the growth and colony formation of *N. sphaeroides* (Deng *et al*., 2008b). These factors significantly influence the structural characteristics of *N. sphaeroides* macrocolonies. Optimal growth is dependent on several key parameters, including light intensity, temperature, pH, nutrient availability, and agitation speed. Moderate light intensity (30–50 µmol photons·m^{-2}·s^{-1}) is essential for promoting photosynthesis while avoiding photoinhibition, which can hinder growth and impact macrocolony formation. Temperature is another essential factor, with an optimal range of 25–30 °C. Deviations beyond this range, particularly below 15 °C or above 35 °C, can slow growth or induce physiological stress, leading to impaired colony development colony development (Deng *et al*., 2008a). Additionally, maintaining pH levels between 7.5 and 8.0 is necessary to support cellular processes and prevent conditions that could disrupt colony integrity. Nutrient availability also plays a fundamental role, particularly nitrogen and phosphorus which are critical for sustained growth. BG 11 medium, commonly used for *Nostoc* cultivation. The light intensity and temperature used in this study (150

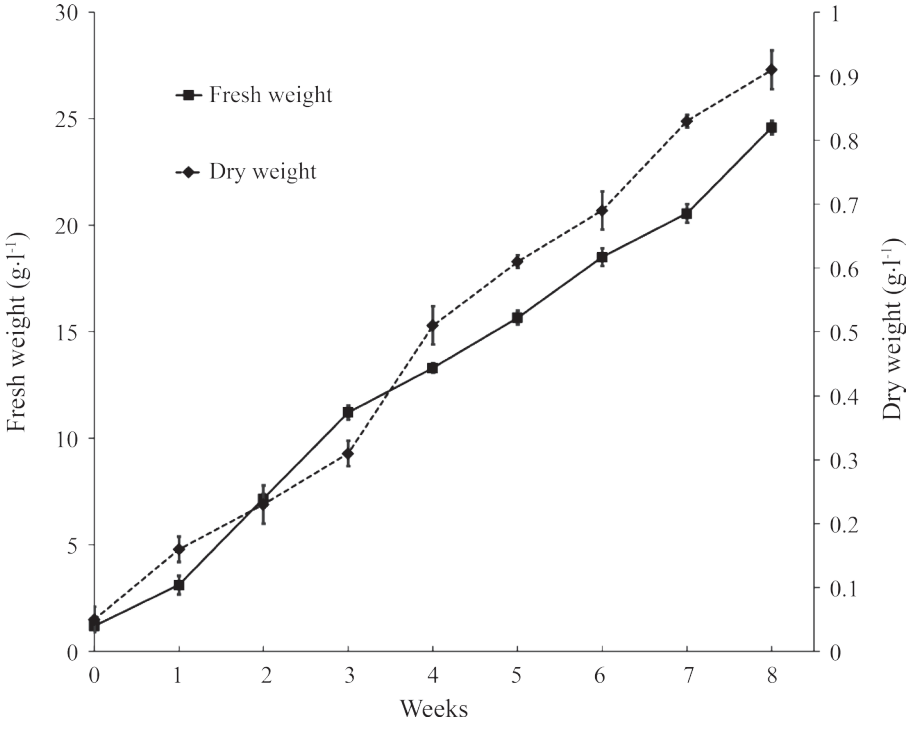

Figure 3. Growth trends of fresh and dry weight (g·L^{-1}) of *Nostoc sphaeroides* grown in BG11 medium culture at 50 rpm for 8 weeks.

Note: Data points represent mean values with standard deviation bars.

μmol photons·m^{-2}·$s^{-1}$and 25±2 °C) were within the optimal range for *N. sphaeroides* growth, as reported in previous studies. Moderate light intensities (30–50 μmol photons·m^{-2}·s^{-1}) are typically sufficient for efficient photosynthesis, while higher intensities, such as 150 μmol photons·m^{-2}·s^{-1}, can further enhance growth without causing photoinhibition. Similarly, maintaining temperatures within the ideal 25–30 °C range ensures optimal photosynthetic activity and colony stability. While these parameters were held constant in this study, future experiments exploring variations in these factors could further refine cultivation conditions for the large-scale production.

As microcolonies develop into macrocolonies, they can grow up to 16 mm in diameter, forming distinct colony layers Gao *et al*. (1998). Deng *et al*. (2008a) demonstrated that filaments in the outer layer of *N. sphaeroides* macrocolonies exhibit higher maximum light saturation points, enhanced photosynthetic rates, and greater overall photosynthetic efficiency compared to those in the inner layer. In contrast, the inner-layer filaments contain higher concentrations of phycobiliproteins and chlorophyll *a*. These differences in light absorption and photosynthetic activity between colony layers are crucial considerations for large-scale cultivation, as they indicate that optimizing environmental factors such as light intensity and nutrient availability could improve both growth rates and the nutritional value of *N. sphaeroides*.

Additionally, agitation speeds between 120–150 rpm have been shown to prevent self-shading and ensures even distribution of light, nutrients, and gases, thereby promoting uniform growth. However, excessive agitation can induce mechanical stress and lead to colony rupture. Therefore, careful control of environmental factors is essential to maximize the growth, structural integrity, and nutritional quality of *N. sphaeroides* in large-scale cultivation systems.

This study provides the first physiological characterization and morphogenesis analysis of a local strain of *N. sphaeroides* in the Philippines. The information gathered is valuable in identifying optimized culture conditions for the large-scale production of this cyanobacterium.

CONCLUSIONS

This study presents the first comprehensive documentation of *Nostoc sphaeroides* cultivation and morphogenesis in local strain of the Philippines. We identified key developmental stages, from single filaments to complex colony structure, including aggregated cell masses (day 7), asteriated colonies (days 13–15), microcolonies (300–350 μm, day 21), and spherical macrocolonies (9–13 mm, day 30). Under optimal conditions of 50 rpm shaking speed, 25±2°C, and a 16:8 light-dark cycle with a light intensity of 150 μmol photons·m^{-2}·s^{-1}, cultures achieved peak biomass yields of 15.672 g·L^{-1} (fresh weight) and 0.610 g·L^{-1} (dry weight) by day 40. These findings offer valuable insights for commercial cultivation of this nutritionally important cyanobacterium. Understanding its morphological transitions facilitates culture monitoring and determination of optimal harvest times. Future research should investigate the effects of additional environmental factors, such as varying light intensity, temperature fluctuations, and media composition, to further optimize cultivation techniques for large-scale production.

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