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Influence of nitrogen on the growth and morphology of cyanobacteria, *Anabaena* sp.

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Abstract. Cyanobacteria are the primary culprits responsible for deteriorating the health of aquatic ecosystems in lakes or rivers. Nitrogen concentration is a crucial factor affecting the synthesis of cellular contents, which, in turn, influence the growth of cyanobacteria. This study focused on the growth and morphological characteristics of *Anabaena sp*., a representative filamentous cyanobacteria, depending on nitrate concentrations varying from 0.5 to 20.0 mg N/L. As the nitrate concentration increased, the filament density, optical density (OD_{680}), cellular protein content proportionally increased. While growing, both nitrate and phosphate concentration depleted, notably affecting the cell compositions in filament: akinete, vegetative, and heterocyst cell. When nitrate concentration was exhausted, the heterocyst cell density of *Anabaena sp*. increased to supplement nitrogen through nitrogen fixation, whereas vegetative cell density kept increasing as long as nitrate remained. Morphological changes in akinete and heterocyst cells in *Anabaena sp*. were less affected by nitrogen concentration, whereas those in vegetative cells were affected by both growth state and nitrogen concentration. The close relationship between heterocyst cells and nitrogen concentration suggests that heterocyst cell can be used to characterize *Anabaena* growth responding to nitrogen loading, as well as provide an important information on nitrogen level affecting *Anabaena* bloom potential.

Keywords: *Anabaena sp*.; cyanobacteria; growth; heterocyst; nitrogen; phosphorus

1. Introduction

Cyanobacteria, also known as blue-green algae, are classified as harmful algae (Cheung and Lee 2013). Cyanobacterial harmful algal blooms (CyanoHABs) result from eutrophication in water bodies, releasing cyanotoxins into the water and eventually deteriorating its quality, and potentially posing a biological hazard to aquatic organisms (Clark *et al*. 2017, Han *et al*. 2022). Over the past few decades, CyanoHABs in freshwater ecosystems have been increasing worldwide, posing a serious threat to drinking water resources, economic sustainability, and ecological balance (Sultana *et al*. 2022). Furthermore, the risks associated with cyanobacterial blooms are expected to escalate due to climate change (O'Neil *et al*. 2012). CyanoHABs are influenced by various factors, including bacterial community composition, nitrogen and phosphorus loads, temperature, and light conditions (Paerl *et al*. 2014, Paerl *et al*. 2016, Choi *et al*. 2024,). To mitigate harmful algae blooms, it is primarily suggested that nitrogen and phosphorus loads entering water bodies should be controlled (Wastson *et al*. 2016).

Phosphorus is recognized as a key limiting nutrient for cyanobacterial bloom, playing a vital role in microalgal growth (Bibi *et al*. 2022). Recent study has also emphasized the need to simultaneously restrict nitrogen loads in eutrophic lakes (Buta *et al*. 2023). Nitrogen has been highlighted as a crucial factor in CyanoHABs due to its impact on microalgal growth, including lipid, protein and carbohydrate synthesis (Yaakob *et al*. 2021). Ammonium, nitrate, nitrite and urea are common nitrogen species that cyanobacteria can assimilate, and variations in nitrogen concentrations elicit different responses in cyanobacterial metabolism and morphology (Herrero *et al*. 2019, Kumar *et al*. 2020). Surface water typically contains excessive levels of nitrate, with nitrate being the dominant nitrogen form originating directly from point sources such as wastewater treatment plants and indirectly through the oxidation of ammonia from nonpoint sources (Cira *et al*. 2016, Hu *et al*. 2021). Elevated nitrate concentrations often lead to severe ecological and environmental issues, including eutrophication, algal blooms, and species extinction in reservoirs, lakes, and rivers (Wurtsbaugh *et al*. 2019).

Cyanobacteria have the potential to utilize nitrate for synthesizing proteins, nucleic acids, co-factors, and secondary metabolites (Norena-Caro *et al*. 2021). Among various cyanobacteria, *Anabaena sp*., also known as *Dolichospermum*, is one of the most frequently occurring cyanobacteria species worldwide (Islam *et al*. 2017, Chia *et al*. 2018). They produce more toxins than other cyano-bacterial groups, including hepatotoxic microcystins, cytotoxic cylindrosporins and neurotoxin toxoid-a (Österholm *et al*. 2020). In particular, these neurotoxins can be harmful to local wildlife, farm animals and pets. Some Do, ichospermum strains also produce geosmin, a volatile odorous metabolite that often causes taste and odor problems in drinking water production (Driscoll *et al*. 2018). This filamentous cyanobacterium has a unique and

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complex cell structure comprising three distinct cell types: heterocyst, akinete, and vegetative cell (Waditee-Sirisattha and Kageyama 2022). In monitoring *Anabaena sp*., it is common to count the entire cells as each cell performs specific roles in their metabolism (Wood *et al*. 2019). The roles of each cell can be summarized as follows: heterocysts fix nitrogen from the atmosphere using their nitrogenase enzyme, akinetes are dormant cells that produce spores under starvation conditions, then germinate and proliferate under favorable conditions, vegetative cells contain chlorophyll and undergo photosynthesis for growth. In addition to cell counting, morphological variations can provide useful information for assessing the growth and physiological status of *Anabaena*. However, to the best of our knowledge, there have been few studies investigating in-depth both the growth and morphology of *Anabaena* depending on nitrate concentration to date.

The goal of this study was to examine the growth and morphological characteristics of *Anabaena sp*., in relation to nitrate concentration. The study assessed the dynamic growth patterns of pure filamentous *Anabaena sp*. and three cells comprising the filaments in terms of their density across four distinct nitrate concentrations. Additionally, morphological changes were examined throughout the growth process by measuring parameters such as length (L), width (W), and the L/W ratio.

2. Materials and methods

2.1 Cultivation of pure Anabaena sp. culture

2.1.1 Preconditioning of pure Anabaena sp. culture

The pure *Anabaena* sp. strain was obtained from the Korea Collection for Type Cultures (KCTC). All glassware was sterilized by autoclaving at 120 °C for 30 min. *Anabaena sp*. was then centrifuged at 5,000 rpm for 15 min, and the settled pellet was resuspended in a nitrogen (N) and phosphorus (P)-free BG 11 medium containing the following constituents per liter: EDTA, 0.001 g, citric acid, 0.06 g, $MgSO_4 \cdot 7H_2O$, 0.075 g, $CaCl \cdot 2H_2O$, 0.036 g, Na_2CO_3, 0.02 g, and 1 mL of trace metal solution. The trace metal solution consisted of $MnCl_2 \cdot 4H_2O$ (1.8 g), $ZnSO_4 \cdot 7H_2O$ (0.22 g), $Na_2MoO_4 \cdot 2H_2O$ (0.39 g), $CuSO_4 \cdot 5H_2O$ (0.08 g), $Co(NO_3)_2 \cdot 6H_2O$ (0.05 g), and H_3BO_3 (2.85 g) per liter. These N and P-starved cells were pre-cultured in an incubator (ED-BIP42, Edun, Korea) for one week under the following conditions: an illuminance of 700 Lux, a 12 h light/12 h dark cycle per day, and temperature of 20 °C.

2.1.2 Cultivation of Anabaena sp. at different nitrogen concentration

The pre-cultured *Anabaena* sp. was transferred to a 250 ml glass conical flask and diluted to an optical density of 0.05 at 680 nm (OD_{680}) using 200 mL of the N and P-free BG-11 culture medium used for preconditioning. According to the Trophic State Classification System, water bodies can be divided into four categories (Lake County Water Atals 2002): oligotrophic (N < 0.4 mg/L), mesotrophic (0.4 mg/L < N < 0.6 mg/L), eutrophic (0.6 mg/L < N < 1.5 mg/L), hypertrophic (N > 1.5 mg/L). Also, nitrate-nitrogen concentrations in many rivers near populated areas have exceeded the recommended healthy water quality standard of 10 mg/L in numerous countries, with levels reported to be up to seven times higher (He *et al*. 2011, Azharpoor *et al*. 2019). In this study, we examined the growth and morphological characteristics of *Anabaena sp*., in response to various nitrate concentration levels. Nitrogen ($NaNO_3$) and phosphorus (K_2HPO_4) sources were added to this BG-11 medium, with nitrate concentrations varying from 0.5 to 20.0 mg NO_3^--N/L while fixing the phosphorus concentration at 1.2 mg PO_4^{3-}-P/L. Each culture sample for a specific N concentration was cultivated in duplicate. Incubation conditions were consistent to those used for preconditioning of *Anabaena* sp. as described in 2.1.1. Each sample flask was manually shaken once a day. Culture samples with different N concentration were collected every three days during the cultivation period to estimating the growth and morphology of *Anabaena* sp. as well as variations in N and P concentrations in the medium.

The specific growth rate of Anabaena sp. for each N concentration was calculated using Eq. (1) (Giannuzzi 2019).

$$\mu_{cd} = \frac{ln\left(\frac{N_t}{N_i}\right)}{T_t - T_i} \quad (1)$$

where, μ_{cd} = specific growth rate (d^{-1})

N_i = filament density of initial *Anabaena* culture (filaments number/mL)

N_t = filament density of *Anabaena* culture at time t (filaments number/mL)

$T_t - T_i$ = elapsed time for incubation (d)

2.2 Morphological analysis

To observe the morphological characteristics of the cultured *Anabaena* sp photographs were taken using a phase-contrast microscope (Primo Star, Zeiss, Germany). The number of *Anabaena* filaments was manually counted using microscopic images captured with a hemocytometer (Petroff-Hausser Counter, Hausser Scientific, USA). A single filament of *Anabaena* sp. was composed of three different cells of akinete, heterocysts, and vegetative cell. The numbers of akinet, heterocyst and vegetative cells were counted for selected *Anabaena* sp. samples. In addition to the number of cells, length (L) and width (W) of three cells in a single filament were analyzed using an image analyzer program (ZEN 3.1 Microscopy Software, Zeiss, Germany). Significant differences in morphological data depending on nitrate nitrogen concentration were statistically analyzed by one-way ANOVA ($p<0.05$ significance levels).

2.3 Analytical methods

The optical densities at 680 nm (OD_{680}) of the culture samples were measured every three-days using a spectrophotometer (DR6000, HACH, USA). Protein contents in *Anabaena* sp. were also measured every three days during cultivation to estimate the changes in assimilated nitrogen into cells according to the Lowry method (Clasics Lowry *et*

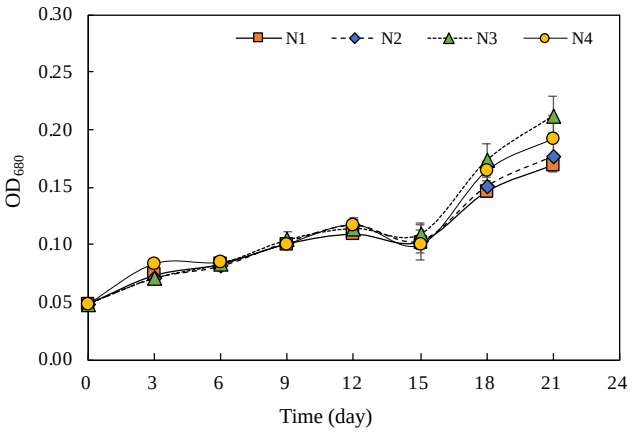
al. 1951). Bovine Serum Albumin (BSA) protein was used as the standard solution, and protein concentrations were determined by measuring absorbance at 650nm using the spectrophotometer. Liquid samples were prepared by filtering the cultivated samples taken every three days by using 0.45 μm syring filter (Whatman, U.K.). Concentrations of NO_3^--N and PO_4^{3-}-P in the liquid samples were analyzed using ion chromatography (Dionex ICS-900, Thermo Scientific, USA).

3. Results and discussion

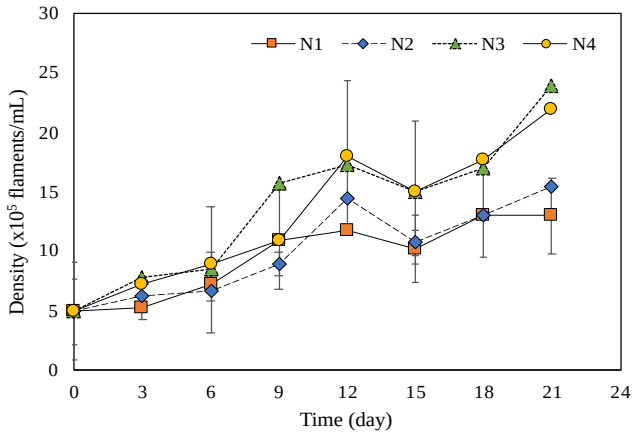
3.1 Effect of N concentration on Anabaena growth

To investigate the effects of N concentration on *Anabaena sp*. cells growth, both OD_{680} and filament density (filament numbers/mL) were measured over a 21-day cultivation period, and the results are depicted in Fig. 1 (a) and (b), respectively. Both OD_{680} and filament densities of *Anabaena sp*. exhibited continuous increases during the cultivation period. Higher initial nitrate N concentration resulted in higher OD_{680} and filament densities of *Anabaena sp*. being measured. The increases in OD_{680} and cell densities were similar across different nitrogen concentrations during the initial period until the 6th day. However, from the 12th day onwards, OD_{680} and filament densities appeared to be noticeably differentiated into two groups based on the initial nitrogen concentration levels (N1 and N2 vs. N3 and N4). Dependency on nitrogen concentration seemed to be more pronounced with filament density than OD_{680}. The accelerated growth of *Anabaena* at higher nitrate concentration levels (N3 and N4) was more closely associated with increased filament numbers, i.e., filament density, possibly through intercalary division of filaments. Proliferation of *Anabaena* can be described by both cell growth (enlargement) and intercalary division, with the latter being more prevalent under favorable conditions (Herrero *et al*. 2016). The highest growth was observed in the N3 culture grown at 10 mg NO_3-N/L, with OD_{680} and filament density reaching 0.2127 and 2.2 × 10^6 filaments/mL, respectively. The specific growth rate for each culture was calculated using Eq. (1), and the results are presented in Table 1. As the N concentration increased, the specific growth rate tended to increase proportionally even though, the maximum specific growth rate was observed at the N3 culture with 0.15 d^{-1}.

While *Anabaena sp*. growing, variations of N and P concentrations in the culture medium are shown in Fig 2 (a) and (b), respectively. The concentrations of N and P steadily decreased due to assimilation by *Anabaena sp*. Particularly, the nitrate concentrations in N1 and N2, with initial nitrate concentration < 2.0 mg N/L, dropped to zero at day 9 and 12, indicating exhaustion of N sources in these cultures. However, interestingly, their concentrations slightly rebounded later and were maintained at an average varying from 0.09 to 0.57 mg N/L until the end of incubation. P concentration declined at a relatively consistent rate regardless of the initial nitrogen concentration. Under N-limited circumstances like N1 and N2, *Anabaena sp*. growth in terms of filament densities tended to be temporarily



(a)



(b)

Fig. 1 Variations of (a) optical density (OD680) and (b) filament density during cultivation at different nitrate nitrogen concentrations

Table 1 The specific growth rate and maximum growth rate of *Anabaena* cultured at four different nitrate N concentrations (N1~N4)

Species	N types	Nitrogen concentration (mg N/L)	Specific growth rate (d^{-1}) (± Standard deviation)	Max. specific growth rate (d^{-1})
Anabaena	N1	0.5	0.05±0.02	0.7
	N2	2	0.05±0.02	0.9
	N3	10	0.09±0.04	0.15
	N4	20	0.09±0.02	0.13

stagnant for 9 and 12 days but recovered their growth rate until the end of cultivation as shown in Fig. 1 (b).

How can *Anabaena sp*. keep growing and utilize P even after nitrogen source exhausted? Under N-rich circumstances (N3 and N4), *Anabaena sp*. grew at a higher rate than in N1 and N2. Even though nitrogen was completely exhausted, *Anabaena sp*. can continue their metabolism and grow by utilizing N supplied through nitrogen fixation. Unlike the other cyanobacteria, *Anabaena sp*. has the ability to grow even at very low N concentrations (> 0.05 mg/L) (Sabour *et al*. 2009). The growth and metabolic availability of *Anabaena* under the external N-limited conditions were

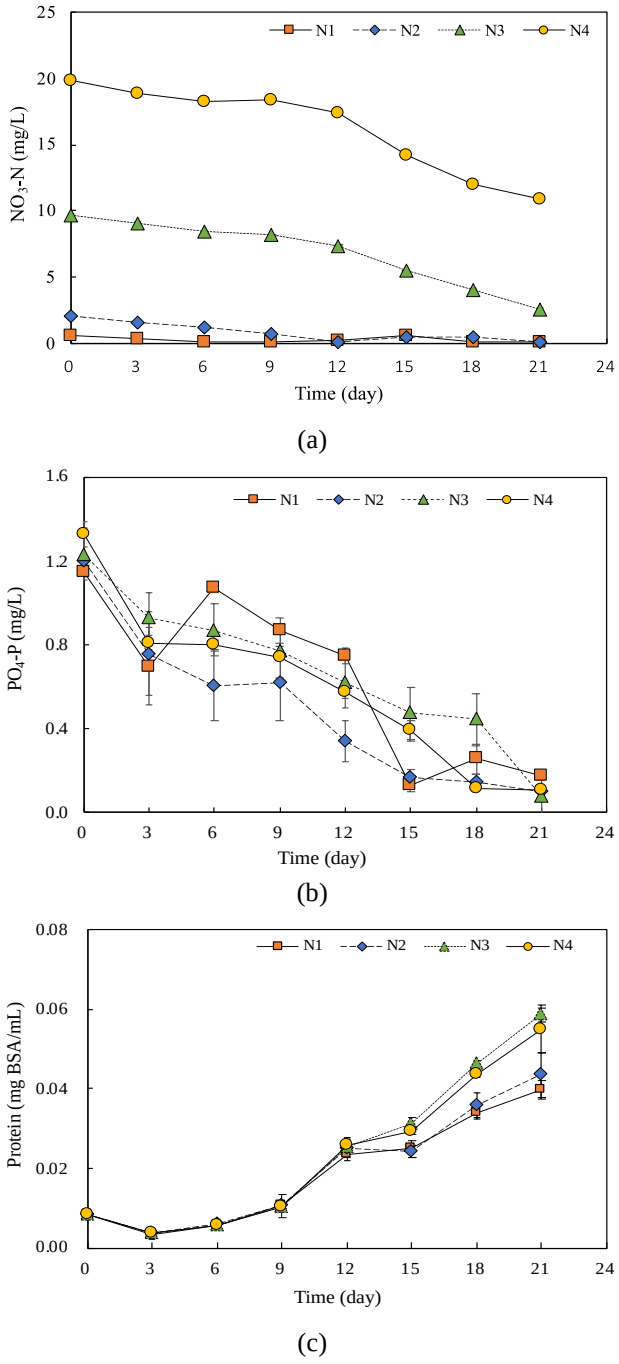


Fig. 2 Variations of the concentrations of (a) N, (b) P, and (c) cellular protein contents during cultivation at different nitrate nitrogen concentrations

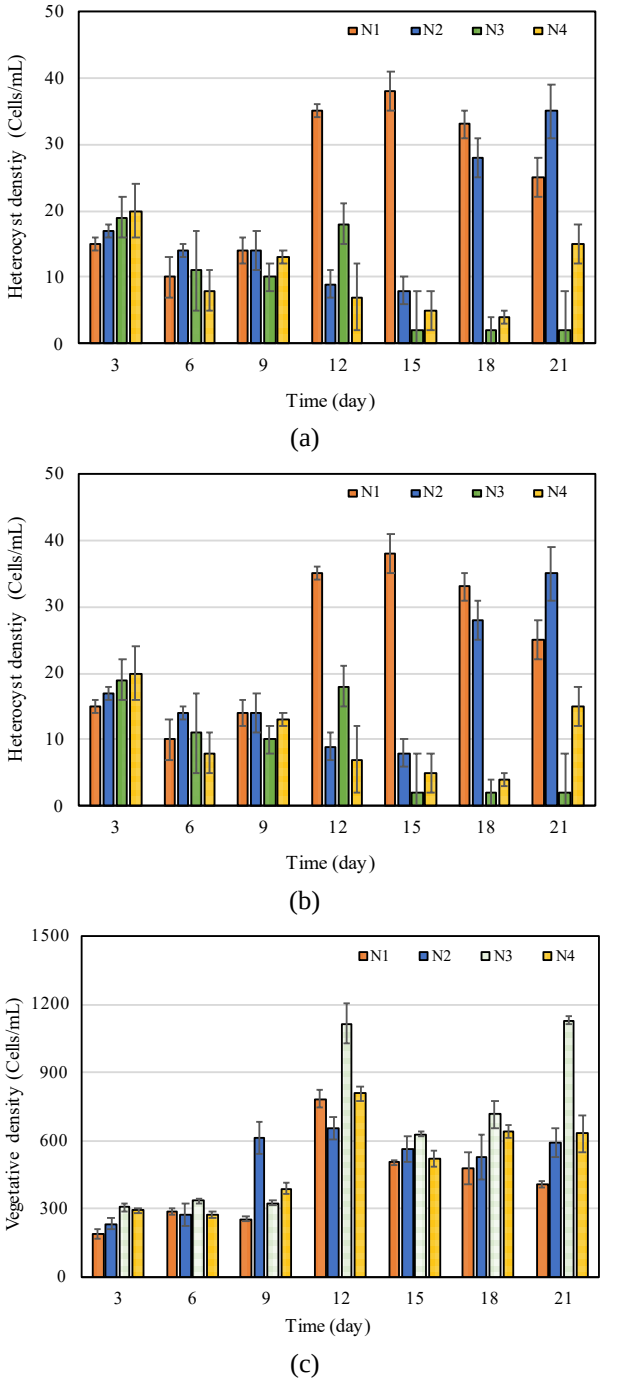


Fig. 3 Changes in density of (a) heterocyst, (b) akinete, and (c) vegetative cells of *Anabaena sp*. for four cultures cultivated with different nitrate nitrogen concentrations

proved by the changes in proteins contents (Fig. 2c). The protein concentrations of all cultures gradually increased until the 12th day. This trend continued until the end of cultivation for N3 and N4 cultures with sufficient nitrogen supply, while the protein contents in N1 and N2 cultures temporarily leveled off after the 12th day, coinciding with the exhaustion of input nitrate. Only three days later, protein synthesis restarted in N1 and N2 cultures, indicating that protein conversion through N fixation took place in these two cultures. It has been clearly demonstrated that a higher N load leads to more protein synthesis in various strains of *Anabaena sp*. (Rosales *et al*. 2016). The results obtained from this study revealed that protein could be synthesized, and cell growth can continue through N fixation even after external nitrogen supply is terminated, due to their unique cell structures with multifunctionality, such as heterocysts and akinetes (Kamshybayeya *et al*. 2023). Their ability to fix atmospheric nitrogen plays a significant role in the nitrogen cycle (Nawaz *et al*. 2024). This capability allows them to dominate in nitrogen-scarce environment and eventually bloom when nitrogen inputs increase (Lakshmikandan *et al*. 2024).

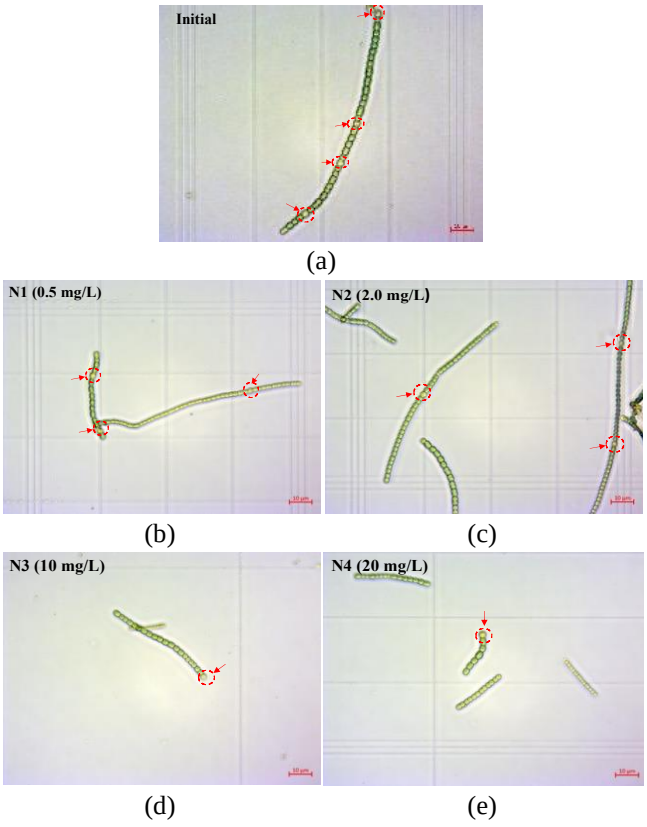


Fig. 4 Microscopic images of *Anabaena sp*. culture (a) before cultivation (initial stage) culture, (b) N1 (0.5 mg N/L), (c) N2 (2 mg N/L), (d) N3 (10 mg N/L), (e) N4 (20 mg N/L). Arrows indicate heterocyst

3.2 Variations of cellular structure of Anabaena depending on nitrogen concentration

A single filament of *Anabaena* consists of three distinct cell types: i.e., heterocyst, akinete, and vegetative cells (Hori *et al*. 2002). In Korea, it is crucial to count these three cells for monitoring *Anabaena sp*. and regulations is based upon the total cell density, which is the sum of the densities of the three cell types, regardless of their specific types. In addition to the growth characteristics, the effects of nitrate concentrations on cellular structures of *Anabaena*, in terms of cell density, were investigated in-depth over 21 days of cultivation (Fig. 3). These three cells could be differently identified in terms of shape and color, which will be in detail described in 3.3.

Variations of the heterocyst cell density notably differed between two groups in terms of initial N concentration: low (N1 and N2) and high (N3 and N4) nitrate N inputs. Heterocyst cell densities in N1 and N2 decreased until the day 6 and 15 respectively, and three days later abruptly increased again as shown in Figure 3 (a). The rebound time for N1 and N2 was the 9th and 18th day, respectively, which completely coincides with the increase in nitrogen concentration in N1 and N2, respectively (Fig. 2(a)). Heterocyst cells proliferate by fixing atmospheric N under N-deficient circumstances (Qin *et al*. 2019), indicating more heterocyst cells at lower nitrogen concentrations (Sabour *et al*. 2009). Overall, *Anabaena* cell growth could be possible under N-deficient conditions by heterocyst cells' dedication of N to vegetative cells. In contrast, heterocyst cell densities in N3 and N4 culture with high N concentration > 10 mg/L, kept decreasing until the end of the cultivation period. When the N source is sufficient, heterocyst cells tend to lose their function for N fixation, while vegetative cells could directly utilize the external N sources for assimilation (Casanova-Ferrer *et al*. 2022). The relative dominance of *Anabaena* compared to other cyanobacteria occurred at a low N/P ratio (< 20) appeared, which was attributed to the contribution by heterocyst for growth under N-limited conditions (Byun *et al*. 2014).

The densities of akinete and vegetative cells varied depending on the nitrate concentration during cultivation period (Figs. 3 b and c). Under harsh and starvation conditions, akinetes deform from vegetative cells and remain dormant, producing spores. Then, they temporarily germinate and proliferate when the environmental conditions change to favorable conditions (Qin *et al*. 2019, Meng *et al*. 2021). As the external nitrate N concentration decreased, akinete densities decreased during cultivation (Fig. 3b). This trend continued in the N-rich cultures (N3 and N4) where both N and P remained in the medium. However, in the N-deficient cultures (N1 and N2), the akinete density increased again on the 12th day and then decreased on the 18th day, indicating that the akinetes underwent repeated germination and proliferation due to changes in N supply from external nitrate N to atmospheric N. The akinete density might fluctuate more prominently when N sources are lacking or changed, even though the exact mechanisms are still unclear. While sufficient nitrogen and phosphorus, especially nitrogen, appeared to be important factors for the germination of akinete in *Anabaena circinalis* (Park *et al*. 2014), this is disputable as some prior studies indicated that nitrogen has an unclear effect on akinete germination and proliferation. Therefore, detailed information on the direct effects of nitrogen on akinetes in Anabaena sp. may require specific experimental studies.

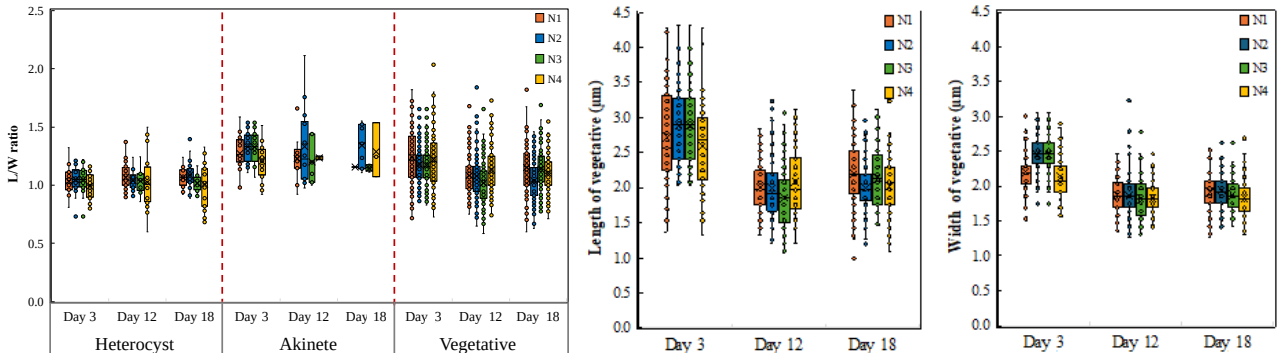
3.3 Morphological variations of Anabaena sp. cells depending on nitrogen concentration

Fig. 4 shows the microscopic images of *Anabaena sp*. in all culture samples taken on day 12. While the three cells comprising a single *Anabaena* filament resemble each other, they can be differentiated by their shape and colors. The heterocyst cell appears as a perfect sphere and is bright yellow. Heterocysts can be further distinguished by their location: intercalary heterocysts are found in the middle of a filament, while terminal heterocysts are located at the edge of a filament as shown in Fig. 4 (Flores *et al*. 2019). In our observation, intercalary heterocyst cells were dominant in N-deficient cultures (initial, N1 and N2), which was likely attributed to the dedication of nitrogen to the neighboring vegetative cells (Fig. 4 a-c) (Xing *et al*. 2022). In contrast, heterocysts in N-rich cultures (N3 and N4) were rarely present within the filament, and the few heterocysts found in these cultures were frequently located at the terminal position (Figs. 4 d and e). The variations of cellular dimension during the overall cultivation period from day 3

Table 2 Length, width, and L/W ratio of vegetative cells, akinetes and heterocyst cells of *Anabaena sp*. samples with different nitrogen concentration (N1~N4) during all incubations (day 3 to day 21).

Morphological characters	Form	N1 (Mean ± SD)	N2 (Mean ± SD)	N3 (Mean ± SD)	N4 (Mean ± SD)
Vegetative	Length	2.22±0.54	2.23±0.58	2.22±0.55	2.19±0.55
	Width	2.00±0.30	2.01±0.34	1.99±0.34	1.93±0.32
	L:W	1.12	1.11	1.12	1.14
	n	701	701	701	701
Heterocyst	Length	2.71±0.38	2.71±0.38	2.73±0.41	2.69±0.37
	Width	2.58±0.38	2.58±0.38	2.63±0.38	2.60±0.35
	L:W	0.90	0.90	1.04	1.05
	n	170	178	115	143
Akinete	Length	3.27±0.55	3.27±0.55	3.58±0.60	3.23±0.49
	Width	2.66±0.36	2.66±0.36	2.80±0.38	2.67±0.34
	L:W	1.24	1.24	1.28	1.21
	n	89	102	46	51

*n represents the number of three types of cells to be analyzed



(a) (b) (c)

Fig. 5 Variation of cellular dimensions depending on N concentration and cultivation period.: (a) L/W ratio of heterocyst, vegetative and akinete cells, (b) length and (c) width of vegetive cells on day 3, 12 and 21

to 21 were analyzed in terms of length (L), width (W) and L/W of each cell, and the results are shown in Table 2. Since cell density including all three cells, is counted for regulation in Korea, and each cell plays a different role in growth and physiology, it is essential to clearly identify each cell through their morphologies, primarily length and width (Wood *et al*. 2019). Based on these dimensions, the size of each cell follows this order: akinete > heterocyst > vegetative cell. The mean length of vegetative, heterocyst, and akinete varied in the narrow range of 2.19 ~ 2.22, 2.69 ~ 2.73, 3.23 ~ 3.58 μm, respectively. Similarly, the mean width of vegetative, heterocyst, and akinete also varied in the narrow range of 1.93~2.01, 2.58 ~ 2.63, 2,66 ~ 2.80 μm, respectively. The L/W ratio of heterocyst ranged from 0.9 to 1.05, describing its shape as a perfect sphere, whereas the other two cells appear rectangular with L/W > 1.1. Akinetes can be clearly differentiated from the other two cells by their larger L, W, and L/W ratio. Heterocysts can be distinguished from vegetative cells by size as well as their colors (bright yellow vs. bright green). It is noteworthy that heterocysts and akinetes are generally larger than vegetative cells, even though specific cell sizes will be affected by environmental factors and culture conditions (Sukenik *et al*. 2015).

Figs. 5a illustrates the variations in the L/W ratio of the heterocyst, akinete, and vegetative cells of the cultures collected on the 3rd, 12th and 18th day, respectively. The L/W ratio of heterocysts and akinetes remained relatively consistent, maintaining values around 1 and 1.2, respectively, indicating that the L/W of these two cells were not highly affected by either N concentration or cultivation time. In contrast, the L/W ratios of the vegetative cells were highly affected by cultivation time, and the difference in the L/W ratios of vegetative cells between day 3 and 12 was statistically significant ($P<0.05$) (Fig. 5a). *Anabaena* growth is typically explained by enlargement and binary fission, i.e., dichotomous growth, of the vegetative cell (Muñoz-García and Ares 2016). Since the number of vegetative cells constitute a predominant portion of the total cell number accounting for 95.3%, it is necessary to further investigate the length and width as well as L/W ratio depending on cultivation time (Figs. 5 b and c). Both the length and width of the vegetative cells tended to decrease as the cultivation time increased. Those on day 3 were the highest regardless

of initial N concentration. Significant decreases in the vegetative cell length and width were observed on the 12th day, indicating that binary fission of cells in all cultures was dominant at this growth stage. This result is well accorded with increases in the vegetative cell densities shown in Fig. 3 (c). Slight increases in the length of N1~N3 with the width fixed were observed on the 18th day, indicating an enlargement of the vegetative cell at this growth stage. Variations in cell length through the repetition of enlargement and binary fission would be greater in the N-rich cultures (N3 and N4) than N-deficient cultures (N1 and N2) because assimilation through direct nitrogen uptake by the vegetative cell is more promoted than through the aid of heterocyst cell's nitrogen fixation (Mullineaux *et al*. 2008). Based on the morphological variations, the size, i.e., principally length and L/W, of vegetative cells were dependent on growth stage, whereas those of heterocysts and akinetes were almost consistent. Since the growth of *Anabaena* was closely related to the nitrogen concentration, the vegetative cell dimension is considered to also be affected by nitrogen concentration. Indeed, the development of innovative monitoring tools utilizing deep learning algorithms presents a promising approach for accurately identifying and counting microalgae (Wood *et al*. 2019), including cyanobacteria such as *Anabaena*. To ensure the effectiveness and accuracy of these tools, it is crucial to provide diverse and precise data regarding morphology and growth characteristics of *Anabaena*.

4. Conclusions

This study elucidates the growth and morphological characteristics of the filamentous cyanobacterium, *Anabaena sp*., in response to varying nitrate nitrogen concentrations. Since *Anabaena sp*. primarily utilizes nitrate for protein synthesis, its growth shows a strong positive correlation with nitrate nitrogen concentrations. However, even under nitrogen-deficient conditions, *Anabaena sp*. can persist and proliferate due to the nitrogen fixation capability of its heterocysts. The density of heterocyst cells increases as external nitrate nitrogen is depleted during cultivation. At this juncture, *Anabaena sp*. enters a developmental phase wherein certain vegetative cell densities rise due to nitrogen dedication by heterocysts. The dimensional attributes of vegetative cells are influenced by both nitrogen concentration and growth stage owing to their dichotomous growth, whereas those of heterocysts and akinetes remain relatively consistent. The detailed insights into *Anabaena sp*. growth and morphology will contribute to the development of robust and reliable monitoring tools for *Anabaena sp*. and other microalgae species, thereby aiding efforts to manage and mitigate the impact of harmful algal blooms.

Acknowledgments

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