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Temperature effects on the growth and morphology of *Anabaena sp*.: lab-scale investigation and onsite validation

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Abstract. This study presents the characteristics of growth and morphology of *Anabaena sp*., a representative filamentous cyanobacterium, depending on temperature variation from 10 to 30 ℃. Both the filament density (or number) and its length of *Anabaena* were highly affected by temperature, as well as growth stage. Rapid growth at a higher temperature led to an increase in *Anabaena* filament density, as well as optical density at 680 nm (OD_{680}). However, the number of vegetative cells within a single filament of *Anabaena* grown at 30 ℃ was smaller than those grown at lower temperatures, due to the intercalary division of the filament. Of the three different cells comprising a single *Anabaena* filament, the vegetative cell marginally affects the growth of *Anabaena*. The main dimensions of the vegetative cell, i.e., length and width, depend on the temperature and growth stage. The length-to-width (L/W) ratios of vegetative cells and akinetes were relatively consistent regardless of the temperature. However, in vegetative cells with dichotomous growth, the L/W ratio shows clear differences depending on their growth stage. It has been demonstrated that the L/W ratio could be used as an indicator to indirectly predict the growth stage of on-sit Anabaena samples.
Keywords: *Anabaena sp*.; cyanobacteria; growth; length; morphology; temperature

1. Introduction

Microalgae, performing oxygenic photosynthesis, are the primary producers of aquatic systems in terms of biodiversity, carbon fixation, and nutrient cycling (Yan *et al*. 2019). Nowadays, they have also been used as bioresources for various beneficial sources of energy, food, and industrial products (Hence *et al*. 2013, Dong *et al*. 2018). However, climate change has promoted the rapid growth of microalgae. Among them, the excess growth of cyanobacteria causes numerous problems in the aquatic ecosystem, such as the deterioration of water quality, as well as the release of cyano-toxin (carpenter *et al*. 1998, Liao *et al*. 2016, Huisman *et al*. 2018). Chlorophyll-a concentration in microalgae has been used over the past decades as a key parameter to monitor the quantity of algal bloom. However, this monitoring parameter causes inaccuracy in an enumeration of cyanobacteria that photo-synthesize with phycocyanin, rather than chlorophyll. Therefore, direct counting methods have recently and widely been used for monitoring algal bloom (Kogure *et al*. 1979), and the World Health Organization (WHO) has established guidance levels for monitoring cyanobacteria in terms of biovolume (mm^3/L) or cell density (cells/L). Accordingly, the Ministry of Environment Korea (MOE) is now operating the algal alert system (AAS) to regularly monitor four harmful cyanobacteria (i.e., *Microcystis*, *Anabaena*, *Aphanizomenon*, and *Oscillatoria*), and to evaluate their quantities in terms of cell density (or number). Experts determine cell density through direct observation, which is highly time-consuming, labor-intensive, and sometimes generates erroneous results. To reduce the human errors of existing methods (microscopic enumeration), it is essential to understand the morphological characteristics of microalgae more accurately (Baek *et al*. 2020).

Most of the microalgal growth is affected by various environmental factors, of which temperature is known to be the most critical factor affecting the growth of cyano-bacteria (Kim *et al*. 2020). The optimal temperature for growth varies among the microalgal type. Cyanobacteria is a mesophilic microalgal group (temperature at 25−35℃), and thereby cyanobacteria generally bloom during summer, rather than the winter season. Temperature can also markedly affect the growth and physiology or morphology of cyanobacteria (Chonudomkul *et al*. 2004). Since the status of cyanobacterial blooming is evaluated by identifying and counting individual cells, it is important to recognize the morphological changes of individual cells depending on various factors in advance. In particular, it can be more crucial for monitoring filamentous-type cyanobacteria (i.e., *Anabaena sp*., *Oscillatoria sp*.), because they have a complex cell structure. For example, a single filament of *Anabaena sp*. contains three different cells:

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heterocyst, akinete, and vegetative cells (Amand *et al*. 2007, Kvíderová *et al*. 2019). These three cells are characterized 1) heterocyst fix the nitrogen from the atmosphere using their nitrogenase enzyme, 2) akinete is a dormant cell that produces spores under harsh and starvation conditions, then germinates and proliferates under favorable conditions, 3) vegetative cells have chlorophyll and grow through photosynthesis.

This unique cell type enables *Anabaena* to survive or proliferate under unfavorable environmental conditions (Graham *et al*. 2016). Even though those cells comprising an *Anabaena* filament have their unique roles, the current enumeration method does not include sorting them due to difficulties in identification, which identification needs to be enhanced by acquiring more morphological data in the future. Many reports in the literature have demonstrated the relationship between microalgal growth and temperature (Giordanino *et al*. 2011, Lind *et al*. 2016, Cha *et al*. 2017). However, to the best of our knowledge, few studies have dealt with dynamic morphological variations of cyanobacteria depending on temperature (Kwak *et al*. 2018, Han *et al*. 2022). Understanding the morphological variation together could provide more comprehensive and deeper information about the growth stage of cyanobacteria and the environmental conditions of the aquatic ecosystem regarding algal bloom.

This study examines the cell growth and morphological characteristics of *Anabaena*, one of the typical filamentous cyanobacteria with a complex structure of filaments, depending on temperature changes. The specific goals of this study are to elucidate the effects of temperature on 1) the growth of *Anabaena* in terms of filament and individual cell density, and 2) dynamic changes in their morphology in terms of length and width during incubation. Also, the dependence of morphological changes on temperature and growth state was validated by investigating those of on-site *Anabaena* samples.

2. Materials and methods

2.1 Cultivation of microalgae

The classified *Anabaena sp*. strain was obtained from the Korea Collection for Type Cultures (KCTC). The strains in 2 L were cultured in a 2 L bottle containing BG−11 culture medium in a shaking incubator at 150 RPM and 20°C (Sneha *et al*. 2023). Cultivated strains in the lab were diluted using the same culture medium (BG−11), until the optical density (OD_{680}) became 0.068. To investigate the effects of temperature on *Anabaena* cell growth, the diluted *Anabaena sp*. cultures were transferred to three 200 mL algal culture bottles, which were separately incubated for 10 d in three separate incubators (ED-BIP42, Edun, Korea) maintained at a constant temperature of 10, 20, and 30°C, respectively. Each incubator provided light at 700 lux from a fluorescent lamp equipped inside for 12 h in a day, maintaining a light/dark cycle. BG−11 culture medium was prepared with the following compositions: ferric ammonium citrate, 0.006 g, EDTA, 0.001 g, citric acid, 0.06 g, $MgSO_4 \cdot 7H_2O$, 0.075 g, $CaCl \cdot 2H_2O$, 0.036 g, $NaNO_3$, 1.5 g, K_2HPO_4, 0.04 g, Na_2CO_3, 0.02 g, and 1 mL of trace metal solution per liter. The trace metal solution contained $MnCl_2 \cdot 4H_2O$ (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.

2.2 Growth characteristics analysis

To investigate the growth characteristics of *Anabaena sp*., the algal cultures grown at each temperature were sampled every two days. Each sample was carefully but quickly taken from the middle of the culture bottle to ensure uniformity in the bottle. The population of *Anabaena* cultures incubated was estimated in terms of both optical density (OD_{680}) and filament density. The OD_{680} was measured by spectrophotometry (DR6000, HACH, USA) at 680 nm, while filament density was determined by counting the number of *Anabaena sp*. filaments in 1 mL aliquot sample through microscopic observation according to Korean water pollution standards (MOE, 2020).

The specific growth rate of *Anabaena sp*. at each temperature was calculated using filament density, as follows (Giannuzzi 2019):

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

where, μ_{CD} = specific growth rate based on filament density (day^{-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)

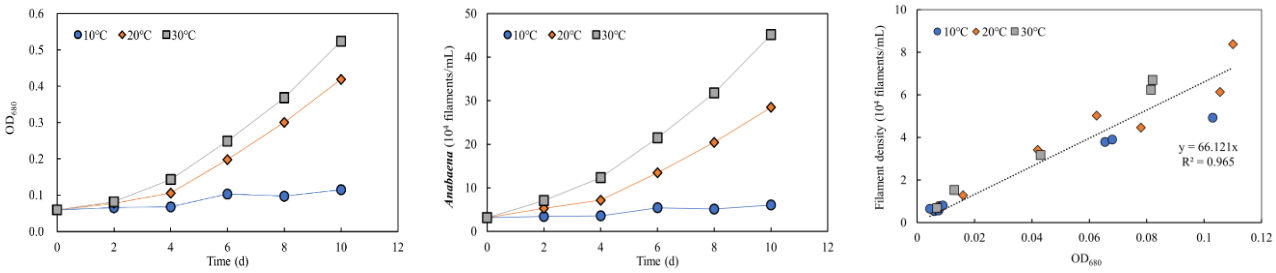
$T_t - T_i$ = elapsed time for incubation (day)

2.3 Morphological analysis

The morphology of *Anabaena sp*. strains was observed using phase-contrast microscopy (Primo Star, Zeiss, Germany). Microscopically photographed specimens in the range of 40− and 200−times magnification were collected from the aliquoted culture media under each condition (i.e., incubation temperature and time). Cells were classified into akinete, heterocyst, and vegetative cells from randomly selected filament images. The number of each cell in a filament was also counted. The dimensions of each cell (length and width) were measured using an image analyzer embedded in the microscope (ZEN 3.1 Microscopy Software, Zeiss, Germany).

2.4 On-site Anabaena sample

On-site cyanobacteria samples were collected from the surface (within 1 m depth) of Daecheong reservoir, which is in the upper reaches of Geum River, Chungcheong province, Korea (36°20’57.7” N, 127°33’36.0” E). Since algal biomass typically blooms in this reservoir from summer to autumn, the samples were taken on July 27, 2021. While sampling, water quality monitoring meters



(a) (b) (c)

Fig. 1 Variations of (a) optical density (OD_{680}) and (b) filament density depending on temperature, and (c) correlation between OD_{680} and filament density

measured environmental factors, such as pH, dissolved oxygen, and temperature (ProDSS, YSI, Germany). Diurnal variation of water temperature data was obtained from the real-time monitoring data of a nearby meteorological station (WEIS, https://water.nier.go.kr/web). The collected samples were preserved in Lugol's iodine solution and transferred to the lab carried in a cooling container for morphological analysis. The morphological characteristics of *Anabaena* in the on-site samples were estimated similarly to that of lab-scale cultured samples.

2.5 Statistical analysis

Microalgae cultivation was carried out in three independent biological replicates, and two technical replicates were performed for each sample analysis. Statistical differences in cell size observed in replicate samples were interpreted using SPSS software (SPSS v12.0 IBM Corporation, Somers, NY). The significance between laboratory data and on-site sample data was verified by one-way ANOVA.

3. Results and discussion

3.1 Effect of temperature on Anabaena growth

To investigate the effects of temperature on *Anabaena* cell growth, both OD_{680} and filament density (filament number per mL) were measured during 10 d of incubation (Figs. 1a and b). The initial OD_{680} and filament density of *Anabaena* cultures were 0.068 and 5,000 filaments per mL, respectively. The OD_{680} of *Anabaena* cultures incubated at 20 and 30°C increased linearly after 4 day (d), and reached 0.42 and 0.53 on d 10, respectively. On the other hand, the OD_{680} of the *Anabaena* culture grown at 10°C increased very slowly, and its maximum value was 0.11 on d 10. In addition to OD_{680}, the filament density of *Anabaena* was estimated by measuring the number of filaments in the samples incubated at different temperatures. Variations in the filament density showed a very similar trend to those of OD_{680}, which kept increasing as the incubation time increased, except at 10°C. The filament density of Anabaena grown at 10°C was almost consistent ($p = 0.359$) during the entire incubation period. In contrast, when *Anabaena* was grown at 20 and 30°C, it increased 9 and 14 times at day 10, respectively.

Table 1 The mean specific growth rate and maximum growth rate of *Anabaena*

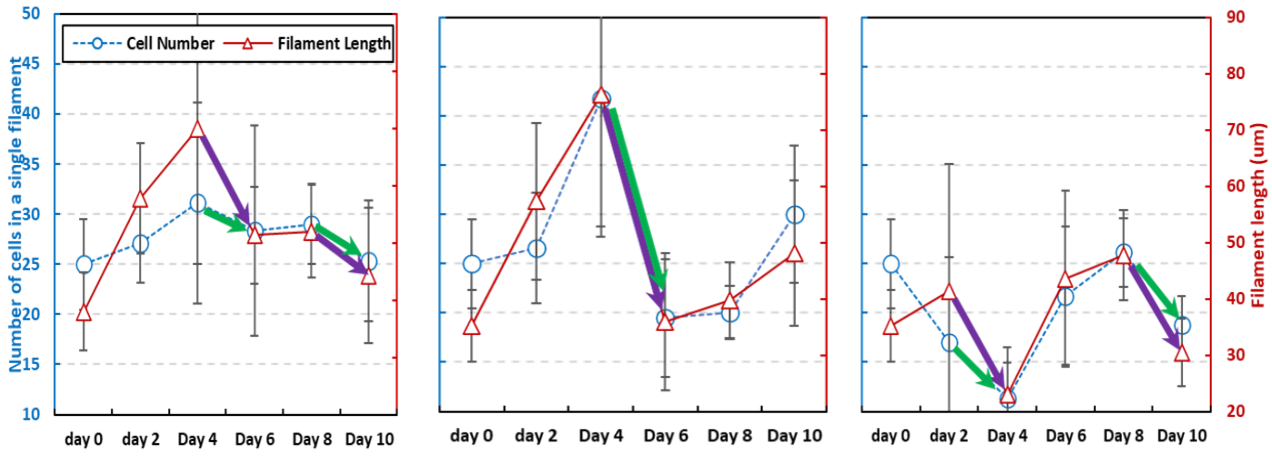
Species	Temp (°C)	Growth rate (day^{-1})		Ref.
		Mean	Max	
Anabaena flos-aquae	10	0.09	0.15	Tomas and Litchman (2016)
	20	0.25	0.31	
	30	0.80	0.90	
Anabaena sp.	10	0.19	0.21	Meng *et al*. (2021)
	20	0.25	0.26	
	30	0.26	0.27	
	10	0.05	0.21	This study

Notably, the OD_{680} value and the filament density of the *Anabaena* culture showed a strong correlation, regardless of the temperature (Fig. 1c). *Anabaena* growth can be described by either enlargement of cell size or proliferation. The latter is more prevalent and is achieved through the dichotomous growth of vegetative cells and intercalary division of filaments (Bornikoel *et al*. 2017). Once the filaments are split by intercalary division, *Anabaena* in the culture medium is predominately composed of short but dense filaments. The strong correlation between the OD_{680} value and the filament density shown in Fig. 1c clearly illustrates that the proliferation via intercalary division of *Anabaena* filament was dominant. This is further discussed in section 3.2.

The maximum specific growth rate of *Anabaena* filaments grown at 20 and 30°C appeared on d 4 and 2, respectively. In contrast, in the sample grown at 10°C, the changes in specific growth rates were negligible. At 10, 20, and 30°C, the maximum growth rate based on OD_{680} was 0.21, 0.31, and 0.41 day^{-1}, respectively (Table 1). The growth rate of *Anabaena* depending on temperature was consistent with those reported in previous studies (Table 1), clearly revealing that the growth rate proportionally increased with the temperature, and that the most vigorous growth occurred at mesophilic conditions.

3.2 Morphological variability of Anabaena depending on temperature

Morphological variations of *Anabaena*, a mesophilic filamentous cyanobacterium, depending on temperature conditions, were investigated. The complex and peculiar



(a) (b) (c)

Fig. 2 Variations of *Anabaena* cell numbers and filament length depending on temperature and incubation time: (a) 10°C, (b) 20°C, and (c) 30°C. Mean ± SD for n = 35

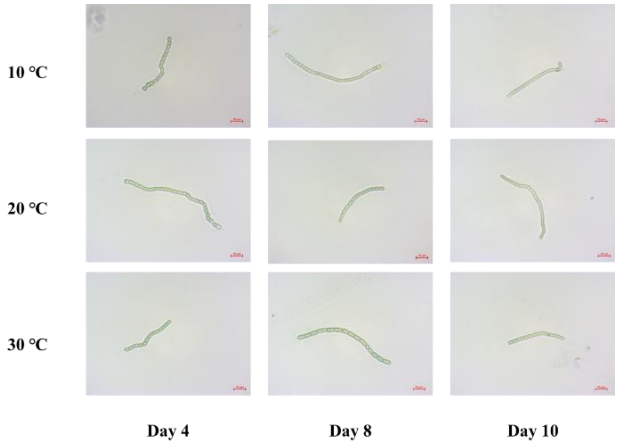


Fig. 3 Microscopic observation of filaments of *Anabaena sp*. strains from cultures incubated at different temperatures (scale bar, 10um, same magnification was used for the three micrographs)

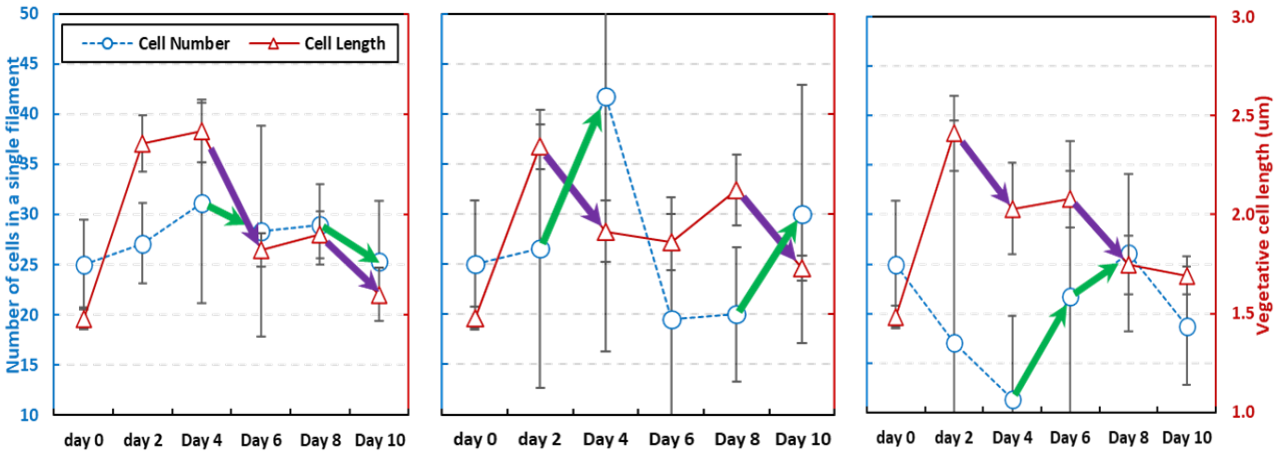
growth of the *Anabaena* can be attained via the following three stages: 1) enlargement of vegetative cells, 2) dichotomous growth of vegetative cells, and 3) intercalary division of the *Anabaena* filament. Once the vegetative cells continue to grow and reach a specific size (length), the vegetative cells separate from the akinete or heterocyst, and then form a daughter filament known as an intercalary division (Rippka *et al*. 1979). Eventually, *Anabaena* growth results from both filament enlargement and filament reproduction. The average size of individual vegetative cells is known to be 1−2 μm, and the entire length of a single filament of *Anabaena* could increase up to 20−30 μm, which is highly dependent on the environmental conditions (Velázquez-Suárez *et al*. 2020).

3.2.1 Variations of Anabaena filament and cell number depending on temperature

In our observation, the initial length of a single *Anabaena* filament was (39.02 ± 6.35) μm, which includes (24 ± 3.5) vegetative cells with (1.41 ± 0.26) μm (length), and (1.14 ± 0.69) akinete with (2.02 ± 0.28) μm (length). In the case of 10 and 20°C incubation, both the filament length and cell number increased during the earlier incubation period. In particular, both the filament length (76.38 ± 25.41) um and cell number (41.75 ± 12.98) doubled at 20°C within 4 d (Fig. 2b). Since the filament length kept increasing during this period, the growth might be close to the dichotomous growth, i.e., cell division, rather than the intercalary division, i.e., filament division, of the filament. Although the growth pattern of *Anabaena* at 30°C was similar to those of 10 and 20°C, variations of growth and morphology were more dynamic (Fig. 2c). Both the filament length and number of vegetative cells appeared to decrease between 2 and 4 d of incubation, illustrating that during this period, dichotomous growth and intercalary division occur almost simultaneously. This phenomenon may not be common, but indicates that growth via either cell division or filament division could occur more rapidly and frequently at higher temperature. Afterwards, these two indicators returned to their initial ranges by 8 days, and then halved again at 10 d. The total length of the filament fluctuated more often and in a lower range than those of the other two temperatures (Fig. 3). Even though the decrease in both cell number and filament length occurred twice (at 5 and 9 d, respectively) in the case of 10 °C incubation (Fig 2a), this is presumed to be associated with cell constriction rather than an intercalary division under an unfavorable temperature condition (Garg and Maldener, 2021). The dependency of vegetative cell number on temperature was probably attributed to the promoted metabolism of vegetative cells at a higher temperature by utilizing more nutrients stored in akinete (Martin-Figueroa *et al*. 2000).

3.2.2 Dimensions of vegetative cells depending on temperature

When *Anabaena* is exposed to a suitable temperature, the vegetative cell grows (enlargement) by utilizing nutrients supplied by akinetes and heterocysts. Also, the individual vegetative cell generates daughter cells within the filament by dichotomous growth. Dichotomous growth



(a) (b) (c)

Fig. 4 Variations of *Anabaena* cell numbers and vegetative cell length depending on temperature and incubation time: (a) 10°C, (b) 20°C, and (c) 30°C. Mean ± SD for n = 35

halving in cell length, prominently occurred on 2−4 and of vegetative cells, i.e., doubling in cell numbers and 8−10 days at 20°C (Fig. 4b). Interestingly, the vegetative cells of *Anabaena* grown at 30°C exhibit more dynamic variations in number and length. At this temperature, the vegetative cell number varied up and down every 4 d, while vegetative cell length kept decreasing after it reached 2.41 µm on day 2 (Fig. 4c). The period from 4 to 8 days can accord with the dichotomous growth of vegetative cells, although for dichotomous growth, the variations of number and length did not precisely coincide. This means that *Anabaena* growth at a high temperature may undergo all three growth phases (enlargement, dichotomous growth, and intercalary division) faster and more frequently than our observation intervals in this study. In other words, it can be challenging to grasp the complex growth pattern of *Anabaena* only with the length information of individual cells. Meanwhile, in the case of *Anabaena* cultured at 10°C, continuous decreases in cell number and length were observed from day 4 onwards (Fig. 4a). Similar to the filament length presented in Fig 2a, constriction in vegetative growth was prevalent throughout the incubation period, rather than either dichotomous growth or intercalary division.

The overall results demonstrated that morphological variations in terms of cell number and length were more dynamic, and three growth behaviors of *Anabaena* occurred simultaneously at a higher temperature. Enlargement and dichotomic growth of vegetative cells increase the *Anabaena* filament length. In contrast, the intercalary division of *Anabaena* filaments limits the filament length but promotes the filament density (Figs. 1b and 2). It is noteworthy that the number and length of vegetative cells of *Anabaena* might be the key control factors for growth responding to environmental conditions, and the supply of nitrogen to vegetative cells by akinete and heterocyst is subject to those environmental conditions (Ishihara *et al*. 2015). These two parameters fluctuated largely at 20°C, where their values were largest at d 4, albeit less frequently than at 30°C. Vegetative cell length varied in the range 1.48 − 2.41 µm, which were identical regardless of temperature.

3.2.3 Comparison of dimensions between vegetative cells and akinete

As described above, the three cells of *Anabaena* play different roles in growth and physiology. Akinete germinates under unfavorable conditions for *Anabaena* growth, while the growth of vegetative cells is promoted through enlargement and dichotomous growth under favorable conditions, i.e., feast and optimal temperature. Therefore, to more precisely understand the growth state of *Anabaena*, it is essential to distinguish vegetative cells and akinetes via morphological manner. Tables 2 and 3 present the dimensions (length and width) of vegetative cells and akinetes as the function of temperature and incubation time, respectively. The morphology of vegetative cell and akinete differs from each other by length rather than width: the width of both cells varied in a similar range from 1.5 to 2.5 µm, while the length of the akinete of 2.0−3.5 µm was noticeably greater than that of the vegetative cells of 1.5−2.5 µm. The length of the vegetative cell increased at the early days of incubation (approximately 2 days), and then repeatedly decreased and increased due to dichotomous growth and enlargement. In contrast, the length of akinete varied in a relatively narrow range, since it is irrelevant to dichotomous growth (Waterbury, 2006). The width of both vegetative cells and akinete increased in the first 2 d, then steadily decreased until the end of incubation, regardless of temperature condition. It is clear that the dichotomous growth of vegetative cells can reduce their width as well as length. Interestingly, it is noticeable that during the growth of *Anabaena*, akinete, which is irrelevant to dichotomous growth, tends to shrink in both length and width as it supplies nutrients to the vegetative cells (Carmago *et al*. 2021).

Figs. 5a and b show the variations of the length-to-width (L/W) ratio of the vegetative cells and akinete, respectively. The L/W ratio did not exhibit any specific relationship with growth stage and temperature condition, albeit slightly lower at 10 °C. The overall mean L/W ratio of vegetative cells varied from 1.0 to 1.3 (Fig. 5a). The temperature effect on the L/W ratio of akinete was insignificant (Fig. 5b). The

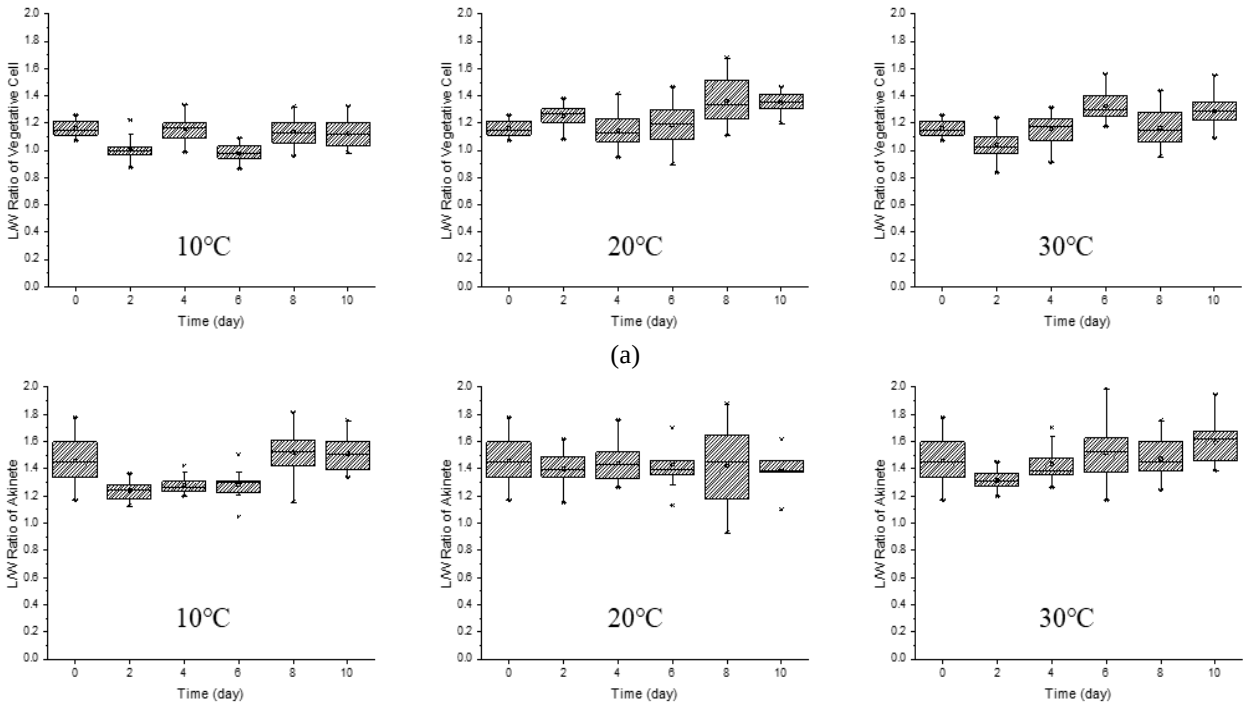


Fig. 5 Variations of cellular dimensions depend on temperature. L/W ratio of (a) vegetative cells and (b) akinetes. n = 35 for vegetative cells and 10-35 for akinetes

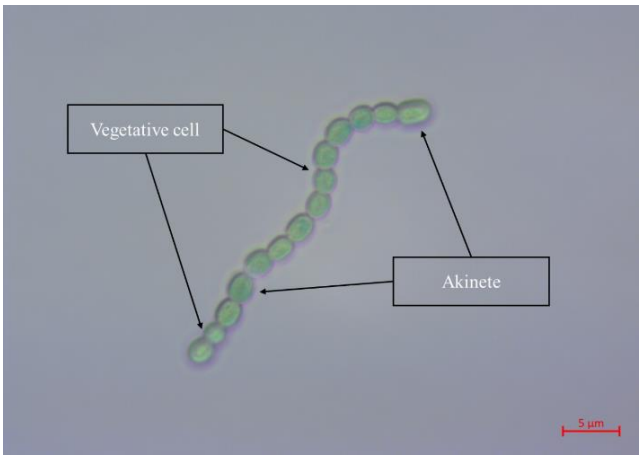


Fig. 6 Vegetative cell and akinete in Anabaena sp. (scale bar, 5um)

L/W ratio of akinete was relatively consistent, varying in a narrow range from 1.23 to 1.61.

Interestingly, the larger L/W ratio of akinete could be a key indicator to distinguish it from vegetative cells and can be effectively used to identify it in an *Anabaena* filament. Otherwise, it is very difficult to differentiate these two cells, because they have similar elliptical shapes (Fig. 6) (Bowange *et al*. 2022). Apart from morphology, the number of akinete decreased substantially at a higher temperature. The number of akinetes observed in filaments dramatically decreased from 1.14 ± 0.69 per filament to 0.49 ± 0.33 per filament. This indirectly indicated that the number of daughter filaments without akinete increased, probably due to the intercalary division of *Anabaena* filaments.

3.3 Statistical analysis for morphological data of Anabaena and comparison with on-site sample

The on-site cyanobacteria samples were compared with laboratory data to predict environmental field conditions. The sampling period was included in a hottest summer in Korea and the ambient temperature was maintained at about 30 °C for a week. Once the on-site *Anabaena sp*. samples were identified, the similarity of morphological traits (i.e., length and width) were compared with laboratory data by one-way ANOVA (Table 2-3).

The morphological traits of *Anabaena sp*. observed from on-site samples showed high similarity in both the length and width of cells cultured at 30°C for 4 d in the laboratory experiment ($p = 0.56$ and 0.66, respectively). The L/W ratio of vegetative cells observed in the field *Anabaena sp*. samples ranged from 0.97 to 1.50 (median value = 1.1), which is similar to the results obtained on d 4 and 8, presumed after the dichotomous growth phase in laboratory experiments of 30°C. These results well-supported the hypothesis that using the morphological traits investigated in this study, it is possible to trace the environmental conditions exposed to *Anabaena*, as well as their growth stage. Indeed, the growth of cyanobacteria can be very different depending not only on species diversity but also on their habitats, such as light intensity, nutrient concentration, water temperature, and salinity (Zhang *et al*. 2018, Velázquez-Suárez *et al*. 2020). It is inferred that automatic monitoring for complex-structured cyanobacteria can be achievable with a higher precision by coupling morphological data with more various ecological influencers.

Table 2 Size distribution of vegetative cells during the incubation period

Temp (°C)	Size (um)	0 d		2 d		4d		6 d		8 d		10 d	
		Length	Width	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width
10	Min	1.403	1.244	2.107	2.204	2.149	1.922	1.706	1.710	1.708	1.541	1.396	1.330
	Max	1.580	1.336	2.744	2.476	2.643	2.249	1.980	2.015	2.094	1.798	1.842	1.553
	Median	1.461	1.265	2.327	2.357	2.443	2.117	1.828	1.862	1.914	1.700	1.597	1.429
	Mean	1.482	1.279	2.352	2.343	2.415	2.095	1.825	1.866	1.899	1.680	1.601	1.428
	SD	0.055	0.029	0.137	0.071	0.155	0.101	0.081	0.097	0.114	0.091	0.130	0.063
	n	35	35	35	35	35	35	35	35	35	35	35	35
20	Min	1.403	1.244	2.124	1.725	1.703	1.507	1.661	1.355	1.805	1.402	1.622	1.201
	Max	1.580	1.336	2.565	2.030	2.197	1.960	2.091	1.884	2.387	1.780	1.845	1.396
	Median	1.461	1.265	2.344	1.851	1.936	1.646	1.889	1.550	2.175	1.578	1.730	1.279
	Mean	1.482	1.279	2.337	1.865	1.915	1.674	1.861	1.587	2.120	1.566	1.731	1.284
	SD	0.055	0.029	0.111	0.093	0.153	0.122	0.139	0.149	0.175	0.108	0.062	0.053
	n	35	35	35	35	35	35	35	35	35	35	35	35
30	Min	1.403	1.244	2.102	2.113	1.666	1.415	1.811	1.440	1.520	1.351	1.512	1.212
	Max	1.580	1.336	2.784	2.556	2.664	2.282	2.299	1.779	1.992	1.771	1.881	1.457
	Median	1.461	1.265	2.397	2.344	2.006	1.750	2.062	1.553	1.771	1.490	1.683	1.314
	Mean	1.482	1.279	2.412	2.329	2.030	1.758	2.078	1.575	1.745	1.502	1.691	1.316
	SD	0.055	0.029	0.187	0.129	0.229	0.210	0.141	0.104	0.147	0.113	0.095	0.080
	n	35	35	35	35	35	35	35	35	35	35	35	35

Table 3 Size distribution of akinetes during the incubation period

Temp (°C)	Size (um)	0 d		2 d		4d		6 d		8 d		10 d	
		Length	Width	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width
10	Min	1.698	1.171	2.208	1.795	2.254	1.823	2.001	1.510	2.018	1.280	2.072	1.305
	Max	2.252	1.554	2.794	2.466	3.245	2.504	2.744	2.255	2.775	1.950	2.830	1.784
	Median	2.030	1.392	2.468	2.056	2.766	2.130	2.256	1.703	2.381	1.563	2.362	1.595
	Mean	1.998	1.373	2.531	2.061	2.738	2.136	2.299	1.800	2.395	1.586	2.360	1.569
	SD	0.171	0.104	0.186	0.192	0.235	0.172	0.203	0.212	0.238	0.170	0.190	0.150
	n	35	35	10	10	23	23	21	21	20	20	15	15
20	Min	1.698	1.171	2.124	1.540	1.738	1.300	2.118	1.367	2.286	1.449	2.092	1.411
	Max	2.252	1.554	3.001	2.530	2.648	1.825	2.858	2.361	2.824	2.649	2.646	1.928
	Median	2.030	1.392	2.702	1.907	2.259	1.539	2.528	1.785	2.493	1.726	2.280	1.719
	Mean	1.998	1.373	2.625	1.891	2.233	1.553	2.499	1.765	2553	1.874	2.336	1.704
	SD	0.171	0.104	0.262	0.261	0.250	0.143	0.233	0.241	0.175	0.398	0.182	0.199
	n	35	35	26	26	28	28	19	19	8	8	5	5
30	Min	1.698	1.171	2.531	1.749	1.771	1.334	2.113	1.366	2.251	1.446	2.111	1.306
	Max	2.252	1.554	3.633	2.774	3.171	2.332	2.954	2.135	2.790	2.065	2.655	1.739
	Median	2.030	1.392	3.189	2.431	2.546	1.701	2.504	1.636	2.537	1.656	2.292	1.444
	Mean	1.998	1.373	3.172	2.419	2.568	1.796	2.490	1.660	2.527	1.728	2.309	1.456
	SD	0.171	0.104	0.298	0.233	0.402	0.313	0.237	0.199	0.166	0.174	0.178	0.130
	n	35	35	25	25	21	21	24	24	18	18	9	9

4. Conclusions

This study investigated the growth and morphological changes of *Anabaena sp.*, a filamentous cyanobacterium, at various temperatures. Numerous external circumstances could determine the growth characteristics of cyanobacteria, but temperature is one of the most prominent factors inducing morphological change. The growth of *Anabaena*

sp. is dominated by the dichotomous growth of vegetative cells and intercalary division of filament. As temperature increases, binary fissions (both vegetative cells and filaments) proceed more rapidly and simultaneously. The rapid growth of *Anabaena sp*. under high temperatures leads to high filament densities, but relatively fewer and shorter vegetative cells in a single filament. Akinete can be differentiated from the vegetative cell with its consistent L/W ratio, regardless of temperature and growth status. These findings may enhance the precision of the manual monitoring of *Anabaena sp*., and furthermore, the gathering and utilization of precise image-related data for *Anabaena sp*. in a deep-learning based online monitoring system in the future.

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