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Effects of environmental factors on the algal organic matters produced by different algal species

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Abstract. Algal blooms due to global warming and eutrophication causes excessive algal organic matters (AOMs) which lead to operational problems in water treatment plant such as unpleasant tastes, odors, and precursors of disinfection by-products (DBPs). In this study, the effects of several environmental factors, including dissolved organic matter (DOM), pH, and suspended solids (SS), on the growth of three different algal species and their AOM production were investigated. The increase in DOM concentration accelerated the growth of all three algal species, including *Anabaena sp*., *Oscillatoria sp*., and Microcystis aeruginosa. Also, AOMs released by these species showed the increase with the DOM concentration. In the case of pH, the growth rates of all three algal species at pH 6.0 were lower than those at both pH 7.5 and 10. Additionally, all algal species under pH 6.0 condition entered the stationary phase earlier than the other pH conditions. An increase in SS concentration was found to negatively affect algal growth by blocking the light necessary for photosynthesis. These findings suggest that environmental factors such as pH, SS, and DOM influence algal-derived organic matters which can cause problems in the water treatment plants. Therefore, it is necessary to understand the physicochemical characteristics of aquatic ecosystem for effective AOM management.

Keywords: algal organic matter (AOM); DOM; pH; SS

1. Introduction

Algal blooms due to global warming and the frequent eutrophication have become a serious environmental issue. The raw water with high algal content can lead to clogging of filters due to particle loading during coagulation and sedimentation processes (Ghernaout *et al.* 2010). Additionally, raw water affected by algal blooms typically contains various algal organic matters (AOMs) including odor, taste, and toxic compounds (Lee *et al*. 2016, Ampiaw *et al*. 2019, Yoon *et al*. 2020).

Generally chemical methods for removal of algae include chlorine, chlorine dioxide, and ozone treatments. These methods were reported to be efficient without additional process (Shen *et al*. 2011). AOM consists of intracellular organic matter (IOM) and extracellular organic matter (EOM) (Pivokonsky *et al*. 2006, Wang *et al*. 2014, Tang *et al*. 2017, Du *et al*. 2022). AOM can block the pores of membranes during filtration, reducing permeability or forming a cake layer on the membrane surface, which leads to inefficient energy consumption in water treatment processes (Ly *et al*. 2017). Photosynthesis in algae-rich water can impact the CO_2 buffering system, leading to changes in pH (Agrawal and Singh 2000, Dnailov and Ekelund 2001). The significant absorption of CO_2, the utilization of carbonates, and the uptake of organic acids by some algae during photosynthesis can raise the pH of water. Conversely, CO_2 produced by algal respiration dissolves in water, producing H^+ ions, which lowers the pH (López-Archilla *et al*. 2004, Vinatea *et al*. 2010). Therefore, pH influences the production of algal species and their associated AOMs (Moheimani 2013).

AOM increases with the frequency of algal blooms and continues to be released even during the death phase of algae. It has been found that the hydrophobic components of algae increase throughout their entire growth phases, while AOM has a higher proportion of hydrophilic components compared to natural organic matter (NOM) (Leloup *et al*. 2013). This finding indicates a shift in the composition of organic matter in the aquatic environment. Unlike NOM, AOM is composed of large biopolymers, including polysaccharides and protein-like substances, and can act as a photosensitizer to enhance the photodegradeability of NOM (Yang *et al*. 2018). This can affect the water quality of rivers and lakes, potentially leading to the generation of toxic by-products.

AOM is known to be a precursor for the formation of disinfection by-products (DBPs) following chlorination (Nguyen *et al*. 2015). Although AOM has a relatively lower potential for DBP formation compared to NOM, the release of IOM due to cell wall destruction during chlorination can cause serious problems in drinking water production by releasing algal toxins and taste and odor compounds (Li *et al*. 2012). IOM contains more high molecular weight proteins than EOM, suggesting that it can form harmful N-DBPs when combined with chlorine and chloramine during

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disinfection processes (Chen *et al*. 2017). Furthermore, EOM and IOM adhere strongly to the surface or porous structure of ultrafiltration (UF) membranes, causing relatively irreversible fouling. This adhesion can be the main cause of UF membrane fouling, emphasizing the need to control algal cell destruction to improve membrane efficiency (Lee *et al*. 2004, Liu *et al*. 2017).

Therefore, it is essential not only to suppress algae in drinking water but also to effectively control algal cell destruction and minimize the release of AOM to prevent the formation of DBPs in water treatment processes. Previous studies have continuously explored methods to suppress AOM release. According to Teixeira *et al*. (2010), the use of dissolved air flotation (DAF) to remove cyanobacteria and suppress AOM release prove effective in removing algae without degrading water quality, making it a promising technology for minimizing IOM release during water treatment. Additionally, Park *et al*. (2019) demonstrated that powdered activated carbon (PAC) effectively removes AOM and reduces the potential for disinfection by-product formation (DBPFP).

Algal blooms are influenced by various environmental factors such as pH, temperature, turbidity, and carbon dioxide concentration, which play crucial roles in the photosynthetic capacity and accumulation of specific metabolites in algal cells. Changes in these factors directly regulate the physiological and ecological responses of algal cells (Nakamura and Miyachi 1982, Juneja *et al*. 2013, Chavan *et al*. 2014, Choi *et al*. 2024). Numerous studies have demonstrated the impact of environmental factors on algal growth and related metabolic activities. Mata *et al*. (2013) showed that algal growth could be either promoted or inhibited by the addition of macronutrients such as magnesium and potassium, as well as micronutrients such as manganese, zinc, and iron, to the culture medium. Wang *et al*. (2015) found that strong light intensity enhanced algal photosynthesis and nutrient uptake, thereby promoting growth. Conversely, Lee *et al*. (2009) reported that high concentrations of dissolved organic matter (DOM) increased algal growth rates. Additionally, hydrophobic DOM had a greater impact on photosynthesis and growth than hydrophilic DOM.

In this study major environmental factors such as pH, SS, and DOM affecting algal growth and the production of algal-derived organic matter were investigated using three different harmful cyanobacteria such as *Microcystis aeruginosa*, *Anabaena sp*., and *Oscillatoria sp*.. This finding can contribute significantly to understanding the complex interactions between algal biological responses and environmental factors, offering crucial insights for research and applications in the control of algal blooms.

2. Materials and methods

2.1 Algal cultivation and extraction of AOMs

In this study *Microcystis aeruginosa*, *Anabaena sp*., and *Oscillatoria sp*. were selected. Research related to these algal species has been widely conducted, and these species are reported as representative harmful cyanobacteria frequently detected during algal blooms in watersheds (Huang *et al*. 2012, Han *et al*. 2022). For the algal cultivation, three major harmful cyanobacteria species were selected. *Microcystis aeruginosa* was obtained from the Freshwater Bioresources Culture Collection (FBCC), while *Anabaena sp*. *and Oscillatoria sp*. were sourced from the Korea Collection for Type Cultures (KCTC). Each species was cultured in BG-11 medium at 140 rpm in a shaking incubator at 23℃-25℃. The components of the BG-11 medium used are listed in Table 1.

Table 1 BG-11 component

Compound	Amount (g/L)
$NaNO_3$	1.5
$CaCl_2.2H_2O$	0.036
Ferric ammonium citrate	0.012
$EDTA.Na_2.2H_2O$	0.001
K_2HPO_4	0.04
$MgSO_4.7H_2O$	0.075
Na_2CO_3	0.02
Trace metal solution[a]	1 ml/L

[a]H_3BO_3,2.86g/L, $MnCl_2.4H_2O$,1.81g/L, $ZnSO_4.7H_2O$,0.222g/L, $Na_2MoO_4.2H_2O$,0.39g/L, $CuSO_2.5H_2O$,0.079g/L, $Co(NO_3)_2.6H_2O$, 0.049g/L.

To prepare EOM solution, algal cultivation solution was centrifuged at 5000 rpm for 15 minutes. After centrifugation, the supernatant was filtered through a 0.45μm glass fiber membrane to obtain the EOM solution (Huang *et al*. 2019). For preparation of IOM, algal cultivation solution was centrifuged at 5000 rpm for 15 minutes. The supernatant was discarded, and the algal cell pellets were washed three times with deionized (DI) water and then frozen at -24°C. The freezing and thawing process were repeated three times to ensure cell lysis. After the final thawing treatment, the sample was carried out by ultrasonic treatment for 15 minutes and was subsequently filtered through a 0.45μm glass fiber membrane to prepare the IOM solution (Li *et al*. 2014).

2.2 Algal cell counting

Algal cell count was conducted using a blood counting chamber according to the method for counting phytoplankton cells (Karampudi and Kamal 2011). An optical microscope (Olympus BX53M, Japan) was employed to visualize the algal cells. The blood counting chamber was divided into upper, lower, and central sections, with sample inlets at the top and bottom where the sample was injected for counting and observation (Fig. 1). Algal cell counts were typically performed by counting the cells in each corner and the central cell of the 25 cells, and the count was then calculated according to Eq. (1).

2.3 Effects of DOM, pH, and SS on algal growth and AOM production

The effects of various environmental factors such as pH, DOM, and SS on algal growth and production of AOMs

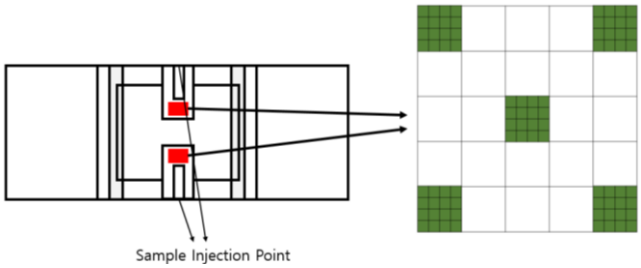

Fig. 1 Blood counting chamber

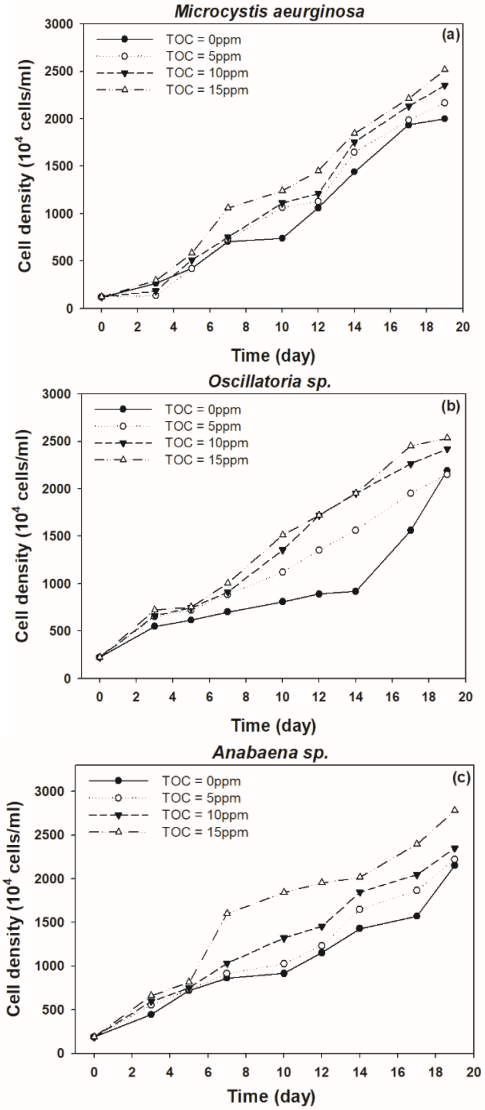

Fig. 2 The effect of glucose on the growth of three algae species: a) *Microcystis aeruginosa*, b) *Oscillatoria sp.*, and c) *Anabaena sp*

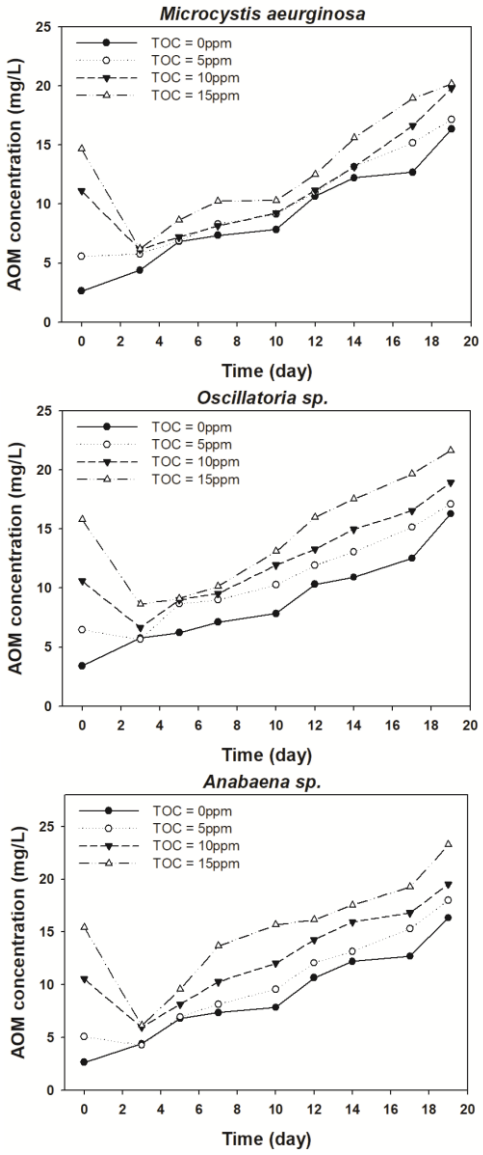

Fig. 3 The effect of glucose on the AOMs produced by three algal species: a) *Microcystis aeruginosa*, b) *Oscillatoria sp.*, and c) *Anabaena sp*

were investigated over a 20-day period. For investigation of the effect of pH, algal growth was observed under various pH values (pH=6, 7.5, and 9). The initial pH values were adjusted by using 0.2M phosphate buffer and 0.1M $NaHCO_3$. The effect of DOM was assessed by adding glucose to the media to achieve initial DOM concentrations of 0 ppm, 5 ppm, 10 ppm, and 15 ppm. DOM concentrations were then measured over a 20-day period using a TOC analyzer (TOC-Vcsh, Shimadzu, Japan). Additionally, different initial concentrations of kaolinite (0 ppm, 5 ppm,10 ppm, and 15 ppm) were prepared to examine the effect of SS on algal growth and AOM production.

3. Result and discussion

3.1 Effect of DOM on algal growth and AOM production

Cyanobacteria can grow using organic carbon compounds, and their growth potential varies depending on the genetic background of specific organisms and other environmental conditions (Tuchman 1996).

Algal cultivation was performed using BG-11 medium containing essential nutrients required for algal growth. To investigate the effects of DOM on algal growth and the

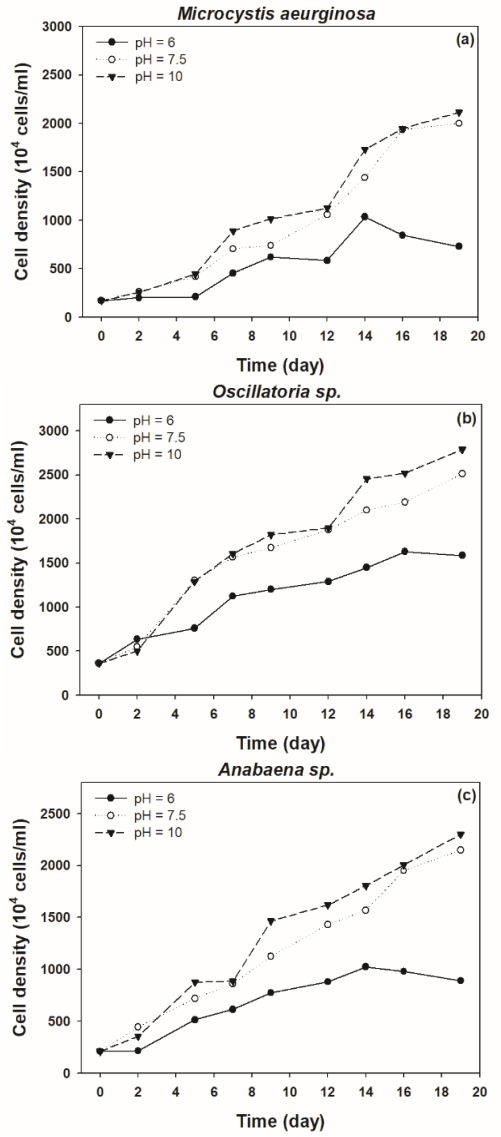

Fig 4. The effect of pH on the growth of three algae species: a) *Microcystis aeruginosa*, b) *Oscillatoria sp*., and c) *Anabaena sp*

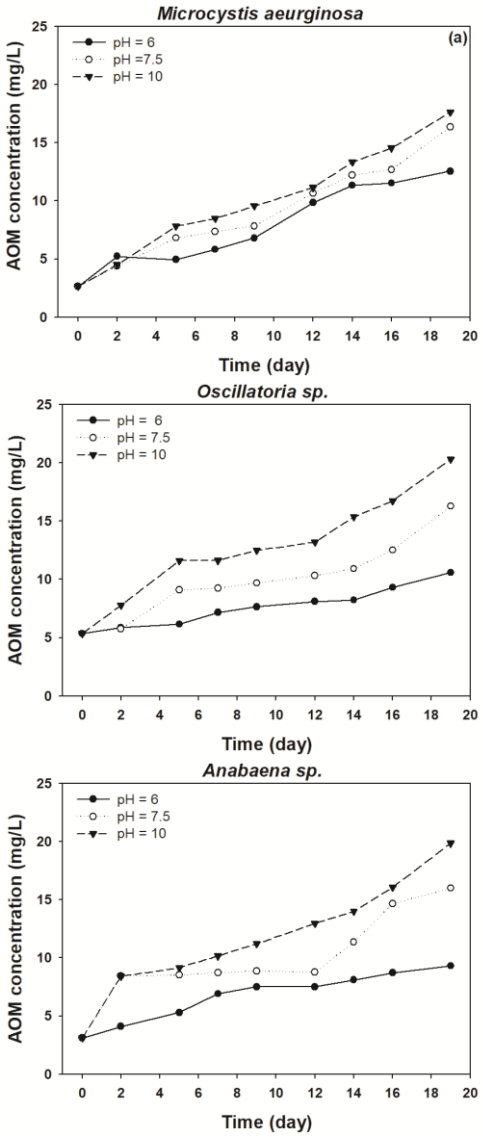

Fig. 5 The effect of pH on the AOM produced by algae: a) *Microcystis aeruginosa*, b) *Oscillatoria sp*., and c) *Anabaena sp*

production of AOM, glucose was additionally introduced into the medium at various concentrations. Fig. 2 shows the effect of DOM on three different algal species and their AOMs. The initial cell concentration of *Microcystis aeruginosa* was 120 x 10^4 cells/mL After 20 days of cultivation, the cell counts of *Microcystis aeruginosa* were 2,000 x 10^4 cells/mL (TOC = 0mg/L), 2,166 x 10^4 cells/mL (TOC = 5mg/L), 2,352 x 10^4 cells/mL (TOC = 10mg/L) and 2,515 x 10^4 cells/mL (TOC = 15mg/L). Compared to the control (TOC = 0mg/L), it was observed that the growth rate of *Microcystis aeruginosa* increased with dissolved organic matter. The initial cell concentrations of *Oscillatoria sp*. and *Anabaena sp*. were 223 x 10^4 cells/mL and 187 x 10^4 cells/mL, respectively. After 20 days of cultivation, similar to *Microcystis aeruginosa*, at TOC = 15 mg/L the highest cell concentrations of *Oscillatoria sp*. and *Anabaena sp*. were 2,000 x 10^4 cells/mL and 2,782 x 10^4 cells/mL respectively. All three cyanobacteria species showed increased growth rates with the increase in dissolved organic matter concentration.

3.2 Effect of pH on algal growth and AOM production

The effect of pH on the growth of three algal species is shown in Fig. 4. The initial cell concentration of *Microcystis aeruginosa* was 170 x 10^4 cells/mL under each pH condition. After 20 days of cultivation, the cell counts of *Microcystis aeruginosa* were 730 x 10^4 cells/mL (pH = 6), 2,000 x 10^4 cells/mL (pH=7.5) and 2,115 x 10^4 cells/mL (pH = 10), respectively. It was observed that the growth rate of algae was higher at pH 10 than at pH 7.5 and pH 6. Additionally, algae mortality was higher under acidic conditions (pH=6.0) than under the alkaline conditions. The result indicates that acidic conditions negatively affected the growth rate of *Microcystis aeruginosa*.

The initial cell concentrations of *Oscillatoria sp*. and

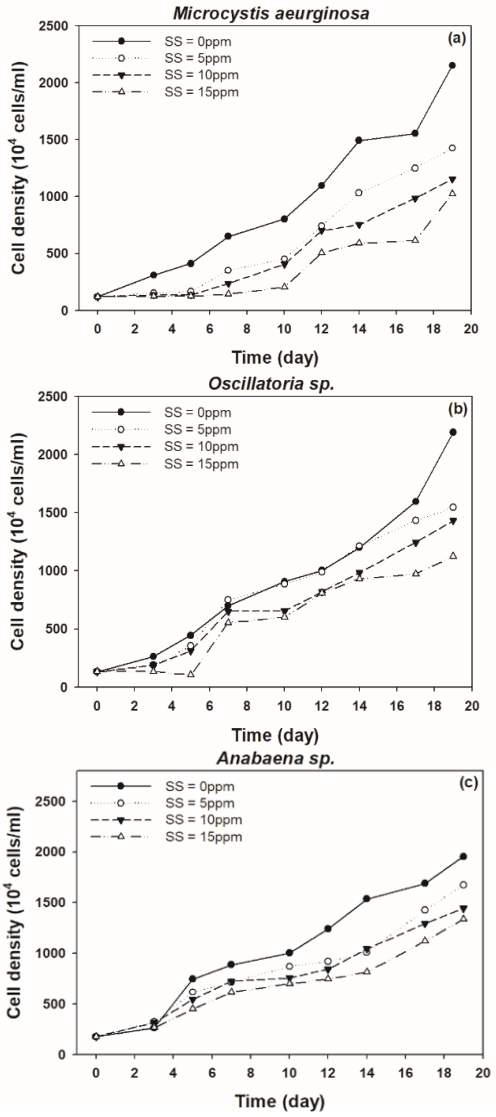

Fig. 6 The effect of SS on the growth of three algae species: a) *Microcystis aeruginosa*, b) *Oscillatoria sp*., and c) *Anabaena sp*

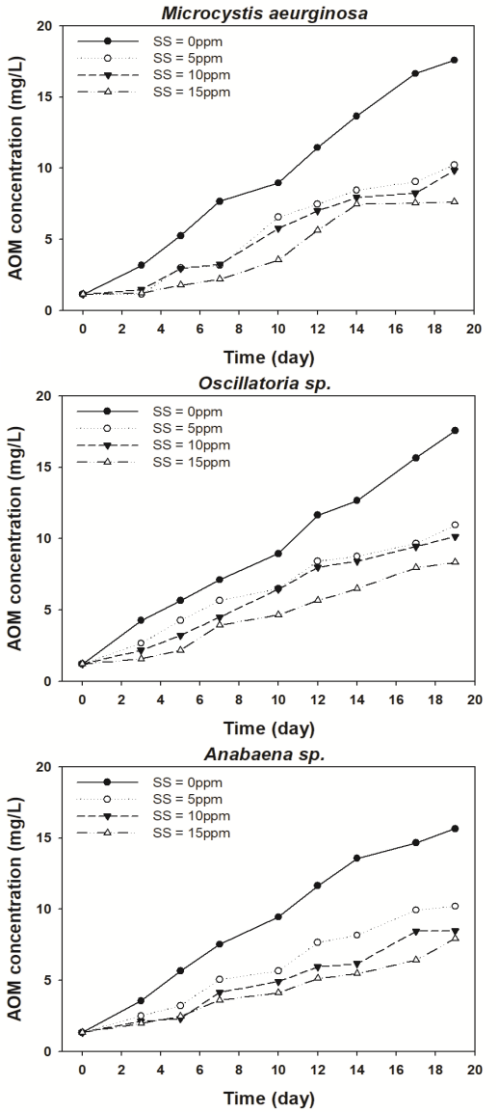

Fig. 7. The effect of SS on the AOM produced by algae: a) *Microcystis aeruginosa*, b) *Oscillatoria sp*., and c) *Anabaena sp*

Anabaena sp. were 360 x 10^4 cells/mL and 210 x 10^4 cells/mL, respectively. After 20 days of cultivation, the cell counts of *Oscillatoria sp*. were 1,585 x 10^4 cells/mL (pH=6.0), 2,515 x 10^4 cells/mL (pH=7.5) and 2,790 x 10^4 cells/mL (pH=10), respectively. For *Anabaena sp*., the cell counts at pH=6, pH=7.5, and pH=10 were 890 x 10^4 cells/mL, 2,150 x 10^4 cells/mL and 2,300 x 10^4 cells/mL, respectively. Similar to *Microcystis aeruginosa*, both algal species showed similar inhibition of growth at pH 6.0. They showed a faster growth rate at pH 10 than at pH 7.5 and pH 6.0. The results were consistent with previous results in the literature (Wangwibulkit *et al*. 2008).

Fig. 5 illustrates the effect of pH on AOMs produced by three algal species. Interestingly, despite the inhibition of algal growth at pH 6.0, the amount of AOM increased over time due to the release of IOM from algal death. It was also observed that the AOM production increased with increasing pH, corresponding to the higher cell concentrations of algae.

3.3 Effect of SS on algal growth and AOM production

SS in aquatic system is a major factor affecting the light availability and primary productivity of algal species (Van Duin *et al*. 2001). The effect of SS on *Microcystis aeruginosa*, *Oscillatoria sp*., and *Anabaena sp*. under various SS concentrations is shown in Fig. 6.

The initial cell concentration of *Microcystis aeruginosa* was 120 x 10^4 cells/mL. After 20 days of cultivation, the cell counts of *Microcystis aeruginosa* were 2,152 x 10^4 cells/mL (SS = 0mg/L), 1,426 x 10^4 cells/mL (SS = 5mg/L), 1,156 x 10^4 cells/mL (SS = 10mg/L) and 1,025 x 10^4 cells/mL (SS = 15mg/L), respectively. Compared to the control (SS = 0mg/L), the growth rate of *Microcystis aeruginosa* increased was relatively low under the other conditions. Similarly, *Oscillatoria sp*. showed a decrease in cell concentration with increasing SS concentrations. In the case of *Anabaena sp*., the cell concentrations decreased with increasing SS concentrations. This result is likely due

to SS in the algae culture system blocking the light needed for photosynthesis (Babin *et al*. 2003, Cao *et al*. 2003).

Fig. 7 shows the effect of SS concentration on the AOMs produced by *Microcystis aeruginosa*, *Oscillatoria sp*., *and Anabaena sp*., respectively. The change in AOM concentration was similar to the changes in cell concentrations for the three algal species. It was observed that the AOMs decreased with the increase in SS concentration.

4. Conclusions

Effects of various environmental factors such as pH, SS, DOM on growth rates of three different algal species and their AOMs were investigated. For this purpose, three representative cyanobacteria such as *Microcystis aeruginosa*, *Oscillatoria sp*., and *Anabaena sp*. were used in this study. All algal species showed increased growth rates with the increase in DOM. Similarly, the amounts of AOMs produced by all the algal species increased with DOM. On the other hand, the growth rates of all algal species increased with pH. Also, the amounts of AOMs produced by all the algal species increased with pH. In the case of SS, the growth rates of all algal species decreased as SS increased. SS seemed to inhibit the growth of algae by blocking light. Investigating the effects of environmental factors could be helpful in understanding algal blooms and their AOM production. Additionally, the results obtained in this study can serve as fundamental data for predicting the concentration of algae-derived organic matter during algal blooms and addressing potential issues in water treatment processes, such as membrane filtration and disinfection. It is also expected that this could be utilized in algae-based biodiesel research.

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