Growth characteristics of *Microcystis aeruginosa* and their effects on coagulation process efficiency

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**ABSTRACT**

In this study, coagulation efficiencies for each growth phase of *Microcystis aeruginosa*, one of the main species involved in eutrophication in South Korea, were examined at the laboratory scale to obtain data on removal characteristics of this organism during the coagulation process. This study also defines the optimum growth conditions to culture *M. aeruginosa* in a laboratory environment. The optimum growth conditions of *M. aeruginosa* were at a concentration of 0.8 mg/L of phosphorus, a concentration of 10 mg/L of nitrogen, 2,000 lx of illumination, and an alkali condition. Coagulation efficiency varies with growth phases, which is due to the different rates of metabolism at each phase. Results provide the optimal growth conditions to obtain *M. aeruginosa* in a laboratory, and characterize coagulation efficiencies at each growth stage for water treatment facilities to establish procedures to manage eutrophicated water.

**Key words** | coagulation dosage, coagulation inhibition, growth characteristics, jar-test, *Microcystis aeruginosa*, UV\textsubscript{254}, UV\textsubscript{260}

**INTRODUCTION**

Water blooms are often caused by nuisance algae and can disrupt water treatment processes by inhibiting the coagulation process, shortening filter longevity, increasing the pH level of the water, and generating unpleasant tastes and odors (Harada & Watari 1992; Daly *et al.* 2007; Takaara *et al.* 2007). In addition, some algae produce toxins which affect human health as well as aquatic organisms and mammals. The main toxin-producing families are blue-green algae such as *Microcystis, Nodularia, Anabaena*, and *Oscillatoria* (Keleti *et al.* 1981; Long *et al.* 1990; Turner *et al.* 1990). Most of the approximately 18,000 reservoirs in South Korea are eutrophicated and water blooms occur during part of the year. The dependence of South Korea on these reservoirs for its water supply suggests potential public health implications as well as economic losses through increased treatment costs for drinking water (Kim *et al.* 2001).

In this study, the impact of different growth phases of *Microcystis aeruginosa* (*M. aeruginosa*) on the coagulation process and the growth characteristics of *M. aeruginosa* cultured in a laboratory environment were investigated. This study focused on *M. aeruginosa* since it is one of the main species of water bloom in South Korea.

**MATERIALS AND METHODS**

*Microcystis aeruginosa*

*M. aeruginosa* used for this study was obtained from the National Institute of Environmental Research (NIER) in South Korea and was cultured with CB medium and M11 medium, as shown in Table 1 (Fujimoto & Sudo 1997; Imai *et al.* 1999; Ueki *et al.* 2004; Chu *et al.* 2007). Table 2 shows the culture conditions for *M. aeruginosa* growth. To count cell numbers during culturing and after jar-testing, 0.1 mL of 12% sodium hypochlorite (NaClO) was added to 100 mL of culture medium and coagulated water samples. After 1 h at room temperature, samples were sonicated with 30 W for 1 min. One-hundredth of a milliliter of a sample was placed in a hemocytometer and...
cell numbers were counted (Huffman et al. 1997; Khoddami et al. 2006).

Growth pattern experiment

To determine optimal growth conditions for *M. aeruginosa*, four physicochemical variables which may affect the growth of *M. aeruginosa* were examined and conditions of the test were listed in Table 2. *M. aeruginosa* were cultured under various conditions (Table 2) and the number of cells was counted daily. All compositions of media for the growth characteristic test were the same as those of the M11 medium except target parameters, i.e. phosphorus concentrations were only adjusted (Table 2) for the test on the effect of phosphorus. Temperature and illumination conditions for media were controlled by a growth chamber.

Jar-test for each growth phase

Coagulation characteristics for each growth phase (lag, log, stationary and death phase) for *M. aeruginosa* were investigated using jar-tests. Water samples for jar-test processes were supplemented with kaolin and CaCO₃ to achieve constant turbidity and alkalinity with the final values being 40 NTU and 100 mg/L, respectively. Uniform aliquots of *M. aeruginosa* culture (2 × 10⁵ cells) were then introduced into 500 mL of a sterile distilled water sample to have the total turbidities of water samples for the jar-test slightly over 40 NTU. The jar-test water was also adjusted to pH 7 ± 0.05 during the whole jar-test process since pH could affect the coagulation processes. Al₂(SO₄)₃·16–18H₂O (Alum) was used as the coagulant. Jar-tests for coagulation characteristics were carried out with 140 rpm (mean velocity gradient, \( G = 51 \text{ s}^{-1} \)) for 10 min to achieve rapid mixing, followed by mixing with 40 rpm (\( G = 7.8 \text{ s}^{-1} \)) for 15 min to allow floc formation. Flocs were settled for 30 min before the test.

Efficiency of coagulation process

To evaluate efficiencies of the coagulation process for removal of *M. aeruginosa* at each growth phase, turbidity, UV₂₅₄, UV₂₆₀, and residual aluminium were analyzed after jar-tests.
using a UV analyzer (Shimadzu UV spectrophotometer 1201, Japan). Ranges for initial values of UV \textsubscript{254} and UV \textsubscript{260} remained between 0.13 and 0.16. Initial values of UV \textsubscript{254} and UV \textsubscript{260} were dependent on the growth phase of the cells. Concentrations of residual aluminium in coagulated water samples were determined by the Atomic Absorption Spectrophotometry Method, Section 2.2.2.11 of Water Quality Standard Methods (Ministry of Environment Republic of Korea 1997).

### RESULTS AND DISCUSSION

#### Growth characteristics of \textit{M. aeruginosa}

**CB medium vs. M\textsubscript{11} medium**

The M\textsubscript{11} medium is usually used in Japan and the CB medium in South Korea, and the study compared those two media for culturing \textit{M. aeruginosa} at the beginning of this research. The growth of \textit{M. aeruginosa} in both the M\textsubscript{11} medium and CB medium showed slightly different patterns. \textit{M. aeruginosa} in the CB medium produced more surface scum and cells aggregated near or at the surface, while cells in the M\textsubscript{11} medium produced less scum and some cells remained suspended in the medium. Cells in the M\textsubscript{11} medium, however, still showed characteristics of cells under natural conditions, and most of the scum was floating on the surface although the scum was thinner than that in the CB medium. Moreover, \textit{M. aeruginosa} cultured in the M\textsubscript{11} medium had a shorter lag phase of two to three days and reached a greater cell density compared to those grown in the CB medium. \textit{M. aeruginosa} cultured in the M\textsubscript{11} medium were chosen for this study because of the high cell yield and shorter time period in reaching maximum cell concentration.

**Effect of phosphorus concentration**

It is crucial to determine the impact of major growth limiting factors such as phosphorus and nitrogen concentrations during eutrophication. Phosphorus is usually available to algae as microorganisms that release PO\textsubscript{4} or extract PO\textsubscript{4} from organic matter which always exist in natural systems. However, the strong relationship between phosphorus concentrations and algal growth makes it one of the most important factors affecting the survival and growth of algae. In fact, dissolved inorganic phosphorus has been considered to be an indirect indicator for phytoplankton concentration in water bodies because the rate of dissolved inorganic phosphorus uptake is the key feature controlling the productivity of phytoplankton (Jacobson & Halmann 1982; Park et al. 1992). Kim et al. (2001) reported that eutrophication events in South Korea were strongly related to high concentrations of phosphorus. Culture conditions except phosphorus were fixed based on the compositions of the M\textsubscript{11} medium for the test to find the effect of phosphorus concentrations. Figure 1(a) shows that \textit{M. aeruginosa} showed a lower growth rate at low phosphorus concentrations (0.02–0.06 P-mg/L). Growth of \textit{M. aeruginosa} rapidly increased at 0.1 P-mg/L and showed similar growth patterns until 4 P-mg/L. The maximum yield of \textit{M. aeruginosa} was revealed when the phosphorus concentration was 0.8 mg/L. The growth of \textit{M. aeruginosa} decreased after 6 P-mg/L.

**Effect of nitrogen concentration**

Since \textit{M. aeruginosa} does not have a nitrogen fixation ability, the study also focused on the concentration of nitrogen in the cultural condition. Optimum nitrogen concentrations for the growth of phytoplankton are usually between 0.3 and 1.5 mg/L, but some require lower or higher concentrations of nitrogen. For example, \textit{Ankistrodesmus falcatus} usually requires a nitrogen concentration greater than 10 mg/L for their maximum growth, while diatoms need less than 1 mg/L of nitrogen concentration. Green algae and blue-green algae need more than 1 mg/L of nitrogen for their growth (Park et al. 1996). Several levels of nitrogen concentrations between 0 and 30 mg/L were tested. Culture conditions except nitrogen were fixed based on the compositions of M\textsubscript{11} medium for the test to find the effect of nitrogen concentrations. Figure 1(b) shows low growth rates when the nitrogen concentration was less than 3 mg/L and the maximum growth was at 10 mg/L. Growth after 10 N-mg/L gradually decreased. Given the results from the phosphorus and nitrogen tests, high concentrations of phosphorus or nitrogen would inhibit the growth of \textit{M. aeruginosa}.
Effect of illumination

The effect of illumination and the photosynthetic rate of algae have been previously reported by several researchers (Konopka & Brock 1978; Tsuji et al. 1994). Blue-green algae actively achieve photosynthesis at a temperature between 20 and 30 °C and the total amount of light in the water body is reduced during the summer seasons because the high density of algae at the surface blocks light penetration (Konopka & Brock 1978). Figure 1(c) shows the result of the illumination test on the growth of *M. aeruginosa* for this study. *M. aeruginosa* did not grow at all without light, and the maximum growth rate was observed at 2,000 lx. The number of cells at 2,000 lx was more than twice those under other light conditions. This result shows that *M. aeruginosa* is sensitive to light intensity and this result may be the key for managing eutrophicated water.

Effect of initial pH

The last experiment on the growth characteristic of *M. aeruginosa* focused on how initial pH levels of the medium affected cell growth. For the pH test, only the initial pH was adjusted because of difficulties of adjusting the pH during the culture period. The initial pH ranged between 6 and 10. The longer lag phase and the lower growth were
shown at pH = 6 and at pH = 7 (Figure 1(d)). *M. aeruginosa* showed much higher growth as pH levels of the medium were increased to alkali conditions, pH > 8. To investigate the effect of acidic conditions of a medium, cells were cultured under acidic conditions; *M. aeruginosa* died off in acidic conditions, under pH = 5, within a few days.

**Coagulation characteristics of *M. aeruginosa* for each growth phase**

**Turbidity changes after jar-test at each growth phase**

Cells of *M. aeruginosa* in each growth phase were examined for coagulation efficiency using jar-tests. The turbidity removal efficiencies for water samples in log growth and stationary phases were lower than those in other phases.

In particular, the removal efficiency of turbidity was higher for water samples with cells in the death phase than in the control sample. Figure 2(a) shows that turbidities after the jar-tests for cells at each growth phase are dependent on coagulant dose. The dashed line with white squares represents the control water sample with no cells.

Takaara *et al.* (2007) reported that substance from excess growth of cyanobacteria may cause reduction of coagulation efficiency and increase coagulant demand. This study showed that coagulation efficiency did not improve after an optimum dose of aluminium (2–4 mg/L). Turbidities for the lag and the death phases stayed below 0.5 NTU after coagulant dose 2–4 mg/L with greater than 98.8% of removal efficiencies (p < 0.05). The jar-tests for cells in log growth and stationary phases also showed the same results, but the final turbidities were slightly higher (0.5–1.8 NTU).

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**Figure 2** | Results of coagulation test of *M. aeruginosa* at each growth phase dependent on coagulant dose. (a) Turbidities after jar-test. (b) Residual aluminium concentrations after jar-test. (c) UV254 after jar-test. (d) UV260 after jar-test.
with 95% removal efficiency ($p < 0.05$). Similar to Takaara et al. (2007), coagulation processes in this study may have been inhibited during the active – log growth and stationary – phases. In other words, cells in the lag phase and death phase may have changes in their surface charge or release different organic matters which could have better reactions with coagulants. In particular, cells in death phase may have characteristics far from those in the other three phases because they have matter from inside of the cells resulting from cell lysis. This hypothesis requires further study.

**Residual aluminium in treated water**

To check the absorption capability of coagulant towards *M. aeruginosa*, residual aluminium concentrations were measured after jar-tests and the results supported the removal rate of turbidity (Figure 2(b)). Concentrations of residual aluminium from the log growth phase and stationary phase were higher than those in the other two phases. Coagulation processes for *M. aeruginosa* in the lag phase and death phase spent more coagulants, so that they reduced the turbidity more than those in the log growth phase and stationary phase. The residual aluminium concentrations at each phase did not show a significant change after an aluminium dose of 6 mg/L. Most of the aluminium might have been spent for the coagulation process since turbidity changes reflect this result as shown in Figure 2(a). Turbidities after aluminium dose of 6 mg/L at each phase were similar. However, further study would be required to prove this hypothesis. Concentrations of residual aluminium were considerably high, between 0.4 and 1 mg/L for most cases. For reference, the Korean drinking water standard for aluminium is under 0.2 mg/L. High concentrations of residual aluminium were expected at higher concentrations of coagulant doses, since the amount of coagulant dosage was high (up to 20 Al-mg/L).

**UV$_{254}$ and UV$_{260}$ of treated water**

Specific UV wavelengths are used to determine the amount of organic matter in water. UV$_{254}$ and UV$_{260}$, which indicate aromatic compounds and humic acids, respectively (Storhoff et al. 2000; Wang et al. 2000; Balcioğlu et al. 2003) were selected for this study. Specifically, UV$_{254}$ is an indirect indicator for trihalomethane (THM) precursors and UV$_{260}$ is an indirect indicator for POPs (persistent organic pollutants) in the water. The study focused on these two UV wavelengths since byproducts of *M. aeruginosa* have been known to contain these organic matter (Jin 2002). Figures 2(c) and (d) show that UV$_{254}$ and UV$_{260}$ in treated water are dependent on coagulant dose. The removal efficiency for these two values was much lower (on average, slightly over 70%) than those for turbidity. The patterns, however, of UV$_{254}$ and UV$_{260}$ in treated water were similar to those of turbidity and residual aluminium concentrations. *M. aeruginosa* in the log growth phase and stationary phase released more organic matter because of their strong metabolism in those phases. The organic matter could affect removal efficiency throughout the entire coagulation process. The results indicate that THMs might be intensively produced during the chlorination process of eutrophicated water if the coagulation process was inhibited in the log growth and/or stationary phases.

**CONCLUSIONS**

The study examined maximum growth conditions for *M. aeruginosa* and the results will be helpful for researchers who study water bloom, water treatment systems for eutrophicated water, and *M. aeruginosa* itself to culture cells in the laboratory. Coagulation characteristics of *M. aeruginosa* revealed different behaviors in each growth phase. The coagulation study showed an optimum range of coagulant dose for *M. aeruginosa* removal. The coagulant should exceed its critical point (2–4 mg/L) to obtain better removal rates, but should not exceed it by too much for the sake of cost efficiency. Residual aluminium concentrations were somewhat high compared to the South Korean drinking water standard (0.2 mg/L), but this is not a critical issue for South Korean drinking water treatment facilities since all facilities now have their own advanced water treatment processes such as activated carbon, ozonization, membrane filtration, after the coagulation process. Facilities, nonetheless, should be careful with coagulant dosage in terms of cost efficiency as mentioned above. The removal efficiency for UV$_{254}$ and UV$_{260}$ values was much lower than those
for turbidity, but patterns were similar to those of turbidity and residual aluminium concentrations. The patterns of cells in the log growth phase and stationary phase reflected greater inhibitions on the coagulation processes than the other phases. The strong metabolism of cells in those two phases could be the reason for coagulation process inhibitions. Drinking water treatment facilities should pay attention to this fact since most cells may be in those two active phases during eutrophication. This study will assist water treatment facilities in developing specific techniques or processes during water bloom to reduce potential risks for public health from cyanobacteria and its byproducts.

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