Effects of different cell states of *Microcystis aeruginosa* on coagulation process

Hun Kyun Bae[†]

Department of Global Environment, School of Environment, Keimyung University, Daegu, South Korea

ABSTRACT

Three different states of *Microcystis aeruginosa*, untreated cells, pure cells, and broken cells, were tested for coagulation efficiency to understand their effects on drinking water treatment facilities. *Microcystis aeruginosa* in its late log-growth phase to early stationary phase, day 12 of culture in the M₁₁ medium, were collected since cells are the most active and reach its maximum population during those periods. Untreated cells samples showed worst results for turbidity removal while turbidities for pure cells and broken cells ones presented the similar levels with those for 0 cell samples after 3 Al-mg/L of coagulant dose. Organic matters from broken cells were not properly removed throughout coagulation processes since samples with organic matters - untreated cells and broken cells - showed higher levels of E₂₅₄ and E₂₆₀ after jar-tests than pure cell samples did. Overall, coagulation processes were highly interrupted when the *Microcystis aeruginosa* cells and organic matters from cells were co-existed. In addition, algae originated matters which may not remove by coagulation process could lead the secondary pollution when algae are introduced into drinking water treatment facilities during waterbloom. Therefore, the facility should pay attention on the results since they have to assure their water quality for public health.

Keywords: Coagulation, Cyanobacteria, Inhibition, *Microcystis aeruginosa*, Waterbloom

1. Introduction

Urbanization and population growth lead increasing input of sewage and wastewater to the waterbodies and result in decreasing water quality, water pollution. Water pollution occurs when contaminants exceeding the capacity of the self-purification of water system and one of typical water pollution is eutrophication. The term "eutropic" is derived from the Greek word "rich in nutrients" and refers to the deterioration of water quality caused by excessive inorganic nutrients and/or organic matters in the waterbodies.

Phytoplankton and algae will dramatically increase once waterbodies are eutrophicated, Especially, cyanobacteria (blue-green algae) will be the dominant strain when temperature is high and this is so called the waterbloom. During waterblooms, cyanobacteria will cover the most of water surfaces and thihs causes blocking oxygen transfer between air and waterbody, perishing aquatic organism as well as generating unpleasant odor and taste. Some cyanobacteria, moreover, produce toxic substances such as hepatotoxin, nevertoxin, and cytotoxin which are causes of dermatitis, stomachache, headache and allergy of human being as well as the death

of aquatic organism and mammals [1-5]. Furthermore, waterblooms by cyanobacteria are the cause of interrupting water treatment process, i.e. inhibiting coagulation process, shortening filter's longevity, increasing pH level, decreasing dissolved oxygen level, etc. [6-8].

Microcystis aeruginosa is the most common strain while waterblooms occur not only in Korea, but also in worldwide. In fact, toxic damages caused by *Microcystis aeruginosa* during waterblooms have been reported worldwide [9-12]. *Microcystis aeruginosa* produces microcystin, one of hepatotoxin, and the toxin may cause severe injury to the liver since it is already reported that hepatotoxins are the cause of the clinical consequences for acute or chronic liver injury, with the possibility of enhanced susceptibility to, and growth of, liver tumors [13]. Furthermore, *Microcystis aeruginosa* inhibit the water treatment process, especially for the coagulation process and filtration process [14-16]. Inappropriate managements, therefore, for *Microcystis aeruginosa* during drinking water treatment process may bring serious public health problems.

In this study, coagulation efficiencies for different algae cell

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[†] Corresponding author

Email: hunkyunbae@kmu.ac.kr

Tel: +82-53-580-5939 Fax: +82-53-580-5164

ORCID: 0000-0002-8067-4923

status was investigated to understand whether coagulation inhibitions would be caused by algae cell itself or by organic matters originated from algae. The results would provide better ideas to water treatment facilities when waterbloom occurs in their water resources.

2. Material and Methods

2.1. Cultivation of *Microcystis Aeruginosa*

Microcystis aeruginosa was obtained from Korean National Institute of Environmental Research and cultured in M₁₁ medium, most widely used for culturing *Microcystis aeruginosa* [17-20]. Table 1 shows the composition of M₁₁ medium. *Microcystis aeruginosa* were collected from its late log-growth phase to early stationary phase, specifically, day 12 of culture when algae reached their maximum cell numbers, approximately 3.4×10^8 cell/mL, since algae in this period should be the most active and release the most abundant organic matters.

Followings are the cell counting process for the study; 0.1 mL of 12% NaClO was added in 100 mL of cultured water as well as coagulated water and left them one hour at room temperature as the pre-treatment. Water samples were sonicated with 30 W for 5 min to make cells fall apart after pre-treatment and then Hemocytometer, broadly used for microcell counting, were used to count cell numbers of *Microcystis aeruginosa* [21, 22].

Table 1. Chemical Composition of M11 Medium

M ₁₁ medium		
Composition	CaCl ₂ · 2H ₂ O	0.004%
	Fe-citrate	0.0006%
	NaNO ₃	0.01%
	K ₂ HPO ₄	0.001%
	MgSO ₄ · 7H ₂ O	0.0075%
	Na ₂ CO ₃	0.002%
	H ₂ O	99.9749%

2.2. Preparing Different Cell States

Three different states of algae cells were prepared for the study; 1. untreated cells 2. pure cells 3. broken cells. Cells in M₁₁ medium were used as they were for untreated cell samples. Untreated cells contain pure cells and External Organic Matters (EOMs). Pure cells were obtained by centrifuging (4,000 g, 15 min) fully grown cells in their late log-growth phase to early stationary phase at M₁₁ medium. The centrifugation was repeated one more time after the supernatant from the first centrifugation was discarded and then samples were filled with distilled water. Broken cells were getting from destroying cell membrane by using high frequency sounds (20 kHz, 100 W, 10 min) and then samples were checked whether or not there might be unbroken cells with microscopic observation. The process was kept repeating until all cells were totally broken. Broken cell samples contain Internal Organic Matters(IOMs), Surface Organic Matters (SOMs), and EOMs [2].

2.3. Jar-Test

500 mL of water samples were used for jar-test. Distilled water was used to rule out the effects of other factors except algae cell itself. Turbidity and alkalinity of water samples were artificially adjusted using Kaolin and CaCO₃; Turbidity 40 NTU and Alkalinity 100 mg/L of CaCO₃. Each state of *Microcystis aeruginosa* were added in water samples after turbidity and alkalinity were adjusted. Jar-tests were carried out with 140 rpm for first 10 min and 40 rpm for following 15 min and then water samples were left for 30 min to settle down flocks. Jar-tests were repeated with every 1 Al-mg increment of coagulant dose, 1 Al-mg to 18 Al-mg, for each state of cell sample and control one. Al₂(SO₄)₃ · 16~18H₂O (Alum) were used as coagulant and pH levels of tested water were adjusted to stay their levels at 7 ± 0.05 using 1 N and 0.01 N of NaOH and HCl during jar-tests.

2.4. Evaluation for Efficiency of Coagulation Process

Turbidity, UV₂₅₄, UV₂₆₀ and residual aluminum were analyzed after each jar-test to evaluate efficiencies of coagulation process for removal of each cell state of *Microcystis aeruginosa* based on Drinking Water Quality Standard Methods [23]. Shimadzu UV spectrophotometer (UV 1800 model) was used to analyze turbidity, UV₂₅₄, and UV₂₆₀. Shimadzu atomic absorption spectrophotometer (AA 6300 model) was used to determine concentrations of residual aluminum in water samples and coagulated water.

3. Results and Discussion

Several factors were analyzed after finishing jar-tests to find coagulation efficiencies for each state of algae cell and followings are the results.

3.1. Turbidity in Jar-Tested Water

Fig. 1 shows jar-test results for turbidity changes of each cell state sample and control dependent on coagulant dose. Control (line with squares) represents 0 cell/L distilled water samples adjusted turbidity and alkalinity.

For controls, a rapid turbidity removal was observed with the first jar-test, 1 Al-mg coagulant dose and the additional removal was shown with the right next jar-test, 2 Al-mg coagulant dose. Turbidity removals for other jar-tests, over 3 Al-mg coagulant dose, were slightly better than that for the second jar-test, 2 Al-mg coagulant dose and stayed in similar levels. Patterns of turbidity removal for broken cell samples were similar to those for control. The results indicate that coagulation process may not inhibit if algae cells are not existed. Jar-tests over 3 Al-mg coagulant dose for pure cell samples had similar turbidity removal rates compared to control and broken cell samples, but jar-tests with 1 and 2 Al-mg coagulant dose for pure cell samples showed worse turbidity removal rates. Pure cells, therefore, may inhibit coagulation processes more than organic matters from *Microcystis aeruginosa* do.

Lastly, turbidities for coagulated water of untreated cell samples showed higher levels than those of other samples although coagulant doses were increased which results showed that coagulation inhibitions were greater when cells and organic matters from

Fig. 1. Turbidity changes for each cell state of *Microcystis aeruginosa* dependent on coagulant dose.

Microcystis aeruginosa coexist rather than cells or organic matters are present singly. The similar result could be found in the previous study which reported that co-existence of algae cells and algae originated matter (AOM) may cause of decreasing coagulation efficiency; 95% of algae cells can be removed by coagulation process while AOMs may not be removed [24]. Treating, therefore, either algae cells or organic matters released from algae might significantly reduce coagulation inhibitions when dealing with water resources during waterbloom.

3.2. Residual Aluminum in Jar-Tested Water

To check the absorption capability of coagulant towards *Microcystis aeruginosa* cell itself or organic matters, residual aluminum were measured after jar-tests and Fig. 2 shows concentrations of residual aluminum in coagulated water for all samples.

Fig. 2. Residual aluminum changes for each state of *Microcystis aeruginosa* dependent on coagulant dose.

Residual aluminum concentrations of coagulated water for control samples showed the lowest levels and those for pure cell samples did the highest as the previous study showed that the increase in algae count and turbidity causes the rise of the concentrations of residual aluminum [25]. The results are considered to be due to the fact that algae cell sizes are much bigger than those of AOMs or kaolin particles. In other words, surface areas of pure cells which could contact coagulants are smaller than those of organic matters or kaolin particles, so that pure cell samples require less coagulants than other samples. Additionally, kaolin particles agglomerate around large algae cells to form larger flocs to settle down which also makes less coagulant consumptions.

Another issue is that residual aluminum concentrations for jar-tests over 3 Al-mg coagulant dose remained at similar levels and same patterns were shown for turbidity reductions. The 3 Al-mg/500 mL of coagulant, therefore, is the optimum dose for turbidity removal and residual aluminum.

3.3. E_{254} and E_{260} of Jar-Tested Water

E_{254} and E_{260} indicate UV_{254} and UV_{260} related substance, respectively. Specific UV wavelengths, UV_{254} and UV_{260} , are used to determine the amount of organic matter in water. E_{254} related to UV_{254} indicates aromatic compounds is an indirect indicator for Trihalomethans (THMs, one of carcinogens) precursors and E_{260} related to UV_{260} indicates for biodegradable organic matters is an indirect indicator for persistent organic pollutants (POPs) in the water [26-28].

The study focuses on E_{254} and E_{260} since byproducts of *Microcystis aeruginosa* have been known to contain these organic matters [2]. Algae cells release the most abundant E_{254} and E_{260} during their log-growth phase and stationary phase rather than their lag phase and death phase and this is another reason why the study focused on cells in log-growth phase and stationary phase since they may affect most on drinking water treatment systems [29].

Fig. 3. E_{254} changes for each cell state of *Microcystis aeruginosa* dependent on coagulant dose.

Fig. 4. E_{260} changes for each cell state of *Microcystis aeruginosa* dependent on coagulant dose.

As mentioned above, AOMs may not be removed although algae cell itself could be eliminated by coagulation process [24]. Pre-treatment such as photocatalytic before coagulation process could increase the removal efficiency for AOMs [30].

Fig. 3 and Fig. 4 show changes of E_{254} and E_{260} in jar-tested water. Changes of E_{254} and E_{260} in jar-tested water for all samples had comparable patterns to each other. It is obvious that samples with organic matters - untreated cells and broken cells - showed higher levels of E_{254} and E_{260} after jar-tests than pure cell samples did. Levels of E_{254} and E_{260} for pure cell samples were even lower than those for controls. As results, SOMs may not related to E_{254} and E_{260} because pure cell samples only have SOMs while others have IOMs and/or EOMs including SOMs. In addition, E_{254} and E_{260} , precursors of THMs and POPs, are more in EOMs than IOMs since both untreated cells and broken cells have EOMs.

4. Conclusions

The study examined coagulation characteristics for different cell states of *Microcystis aeruginosa*, most common species during water blooms, to give better ideas for researchers, managers and operators who focused on drinking water treatment facilities when waterbloom occurs on their water resources. Coagulation characteristics of *Microcystis aeruginosa* revealed different behaviors in each cell state.

For the single existence of pure cells or organic matters, broken cell samples showed better turbidity removal rates than pure cell samples did, so pure cells may inhibit coagulation processes more than organic matters from *Microcystis aeruginosa* do. Turbidities for coagulated water of untreated cell samples showed higher levels than those of other samples in all jar-tests with different coagulant doses which results showed that coagulation inhibitions were greater when cells and organic matters coexist rather than cells or organic matters are present individually. Treating, therefore, only one of

algae cells or organic matters released from algae might significantly reduce coagulation inhibitions when waterbloom occurs in water resources.

Pure cell samples showed the highest concentrations for residual aluminum after jar-test. Facts that algae cells are bigger than algae-derived organisms or kaolin particles and kaolin particles agglomerate around large algae cells to form larger flocs to settle down makes less coagulant consumptions.

Also, residual aluminum concentrations after jar-tests were inversely proportional to turbidity removal rates, but their patterns were analogous; their concentrations remained in similar levels for jar-tests over 3 Al-mg coagulant dose. The optimum coagulant dose, therefore, for turbidity removal is 3 Al-mg/500mL, so the coagulant dose should exceed its critical point to obtain better removal rates, but should not exceed it by too much for the sake of cost efficiency.

The removal patterns for E_{254} and E_{260} of all cell states samples were similar. Samples with organic matters showed higher E_{254} and E_{260} and levels of E_{254} and E_{260} for pure cell samples were even lower than those for controls. SOMs, therefore, may not related to E_{254} and E_{260} and EOMs may be the main sources of E_{254} and E_{260} since both untreated cells and broken cells have EOMs. The results indicate that water treatment facilities should pay special attentions to EOMs, so that they could remove potential risks for public health from *Microcystis aeruginosa* and its byproducts. Moreover, it is better to remove organic matters rather than algae cell itself in order to reduce the coagulation inhibition since organic matters could have potential risks to the public health.

The study will assist water treatment facilities in developing specific techniques or processes during waterbloom to reduce potential risks for public health as well as water treatment process inhibitions from cyanobacteria and its byproducts.

Author Contributions

H.K.B. (Professor) conducted all experiments and analyzed all data and results.

References

1. Liu Q, Zhang G, Ding J, Zou H, Shi H, Huang C. Evaluation of the Removal of Potassium Cyanide and its Toxicity in Green Algae (*Chlorella vulgaris*). *Bull. Environ. Contam. Toxicol.* 2018;100:228-233.
2. Jin JS. The effective removal of *Microcystis aeruginosa* and its by-products in drinking water treatment plants [dissertation]. Daegu: Keimyung University; 2002.
3. Turner PC, Gammie AJ, Hollinrake K, Codd GA. Pneumonia associated with contact with cyanobacteria. *BMJ.* 1990;300:1440-1441.
4. Long EG, Ebrahimzadeh A, White EH. Alga associated with diarrhea in patients with acquired immunodeficiency syndrome and travelers. *J. Clin. Microbiol.* 1990;28:1101-1104.
5. Keleti G, Sykora JL, Maiolie LA, Doerfler DL, Campbell IM. Isolation and characterization of endotoxin from cyanobacteria

- (blue-green algae). The Water Environment: Algal Toxins and Health, W.W. Carmichael (Editor), 1st ed. Springer; 1981. p. 447-464.
6. Buratti FM, Manganelli M, Vichi S, et al. Cyanotoxins: producing organisms, occurrence, toxicity, mechanism of action and human health toxicological risk evaluation. *Arch. Toxicol.* 2017;91:1049-1130.
 7. Takaara T, Sano D, Konno H, Omura T. Cellular proteins of *Microcystis aeruginosa* inhibiting coagulation with poly-aluminum chloride. *Water Res.* 2007;41:1653-1658.
 8. Daly RI, Ho L, Brookers JD. Effect of chlorination on *Microcystis aeruginosa* cell integrity and subsequent microcystin release and degradation. *Environ. Sci. Technol.* 2007;41:4447-4453.
 9. Yeom HS, Son H, Ryu JS, Jung EY, Kim KA. Release of Microcystin from *Microcystis* sp. by Pre-Oxidation and Removal of Microcystin by Advanced Drinking Water Treatment. *J. Korean Soc. Environ. Eng.* 2019;41:532-540.
 10. Kim IS, Nguyen GH, Kim S, Lee J, Yu HW. Evaluation of Methods for Cyanobacterial Cell Lysis and Toxin (Microcystin-LR) Extraction Using Chromatographic and Mass Spectrometric Analyses. *Environ. Eng. Res.* 2009;14:250-254.
 11. Luukkainen R, Sivonen K, Namikoshi M, Färdig M, Rinehart KL, Niemelä SL. Isolation and identification of eight microcystin from thirteen *Oscillatoria agardhii* strains and structure of a new microcystin. *Appl. Environ. Microbiol.* 1993;59:2204-2209.
 12. Sivonen K, Namikoshi M, Evans WR, et al. Isolation and characterization of a variety of microcystins from seven strains of the cyanobacterial genus *Anabaena*. *Appl. Environ. Microbiol.* 1992;58:2495-2500.
 13. Falconer IR, Burch MD, Steffensen DA, Choice M, Coverdale OR. Toxicity of the blue-green alga (cyanobacterium) *Microcystis aeruginosa* in drinking water to growing pigs, as an animal model for human injury and risk assessment. *Environ. Toxicol.* 1994;9:131-139.
 14. Yang B, Park JA, Nam HL, et al. Degradation of Microcystin-LR, Taste and Odor, and Natural Organic Matter by UV-LED Based Advanced Oxidation Processes in Synthetic and Natural Water Source. *J. Korean Soc. Environ. Eng.* 2017;39:246-254.
 15. Li S, Tan H-Y, Wang N, et al. The Role of Oxidative Stress and Antioxidants in Liver Diseases. *Int. J. Mol. Sci.* 2015;16:26087-26124.
 16. Cadel-Six S, Moyenga D, Magny S, Trotureau S, Edery M, Krys S. Detection of free and covalently bound microcystins in different tissues (liver, intestines, gills, and muscles) of rainbow trout (*Oncorhynchus mykiss*) by liquid chromatography-tandem mass spectrometry: Method characterization. *Environ. Pollut.* 2014;185:333-339.
 17. Chu Z, Jin X, Iwami N, Inamori Y. The effect of temperature on growth characteristics and competitions of *Microcystis aeruginosa* and *Oscillatoria mougeotii* in a shallow, eutrophic lake similar system. In: Qin B, Liu Z, Havens K, eds. *Eutrophication of Shallow Lakes with Special Reference to Lake Taihu, China. Developments in Hydrobiology*, vol 194. Dordrecht: Springer; 2007. p. 217-223.
 18. Ueki M, Matsui K, Choi K, Kawabata Z. The enhancement of conjugal plasmid pBHR1 transfer between bacteria in the presence of extracellular metabolic products produced by *Microcystis aeruginosa*. *FEMS Microbiol. Ecol.* 2004;51:1-8.
 19. Imai A, Fukushima T, Matsushige K. Effects of iron limitation and aquatic humic substances on the growth of *Microcystis aeruginosa*. *Can. J. Fish. Aquat. Sci.* 1999;56:1929-1937.
 20. Fujimoto N, Sudo R, Sugiura N, Inamori Y. Nutrient-limited growth of *Microcystis aeruginosa* and *Phormidium tenue* and competition under various N:P supply ratios and temperatures. *Limnol. Oceanogr.* 1997;42:250-256.
 21. Khoddami M, Farzaneh M, Anaraki MG. Diagnosis of urinary tract infection using standard urinalysis or Hemocytometer leukocyte count. *Iran. J. Pathol.* 2006;1:117-120.
 22. Huffman PA, Arkoosh MR, Casillas E. Characteristics of peripheral blood cells from rainbow trout evaluated by particle counter, image analysis, and Hemocytometric techniques. *J. Aquat. Anim. Health.* 1997;9:239-248.
 23. KME (Korea Ministry of Environment). Drinking water quality standard methods. Announcement of Ministry of Environment, Korea, Announcement No. 2009-233. 2010.
 24. Zhao Z. Effects of Drinking Water Treatment Processes on Removal of Algal Matter and Subsequent Water Quality. [dissertation]. Ontario: The University of Western Ontario; 2020.
 25. Mohamed FM, El-Deen FN, Kamal AM. The Relationship between Algal Counting and Chemicals Consumption of Conventional Purification Systems at Qena Governorate, Egypt. *Egypt. J. Aquat. Res.* 2020;24:161-172.
 26. Balcioğlu IA, Alaton IA, Otker M, Bahar R, Bakar N, Ikiz M. Application of Advanced Oxidation Processes to Different Industrial Wastewaters. *J. Environ. Sci. Health* 2003;38:1587-1596.
 27. Storhoff JJ, Lazarides AA, Mucic RC, Mirkin CA. What Controls the Optical Properties of DNA-Linked Gold Nanoparticle Assemblies? *J. Am. Chem. Soc.* 2000;122:4640-4650.
 28. Wang G-S, Hsieh S-T, Hong C-S. Destruction of humic acid in water by UV light-catalyzed oxidation with hydrogen peroxide. *Water Res.* 2000;34:3882-3887.
 29. Lee TG, Bae HK. Growth characteristics of *Microcystis aeruginosa* and their effects on coagulation process efficiency. *Wat. Sci. Tech.: Wat. Sup.* 2012;12:124-131.
 30. Qi J, Lan H, Liu R, Liu H, Qu J. Efficient *Microcystis aeruginosa* removal by moderate photocatalysis-enhanced coagulation with magnetic Zn-doped Fe₃O₄ particles. *Water Res.* 2020;171:115448.