Production of a Serious Musty Odor in the Clean Upper Reaches: Behavior of the 2-MIB Production by Benthic Cyanobacteria and Our Countermeasures

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Abstract
Recently, the concentration of 2-MIB has been increased in the clean upper reaches in the Tama River, being a drinking water resource of the Tokyo Metropolis, due to the growth of 2-MIB producing cyanobacteria adhered to riverbeds. Therefore, we investigated the actual situation of the concentration of 2-MIB and the growth of 2-MIB producing cyanobacteria in the river. As a result, we found that 2-MIB producing cyanobacteria were able to grow in both winter and summer, whereas the 2-MIB production tended to be affected by the water temperature. We verified that the water temperature lowering suppressed 2-MIB production by the culturing experiment of 2-MIB producing cyanobacteria, whereas the cell densities of the cyanobacteria eventually reached at the same level. We also carried out the experiment to introduce the intermediate chlorination instead of the prechlorination to Water Purification Plant being affected by 2-MIB at a pilot plant. After starting the full-scale operation, the removal effect of 2-MIB on powdered activated carbon treatment was improved to approximately twice. In conclusion, the study revealed that we were able to take countermeasures against 2-MIB produced by benthic cyanobacteria in the clean upper reaches of resource river for drinking water.

Keywords
2-methylisoborneol; benthic cyanobacteria; intermediate chlorination; musty odor; *Phormidium*; powdered activated carbon

INTRODUCTION

Foul odors that affect the taste of tap water constitute are the major issue for Japanese water utilities. 2-methylisoborneol (hereinafter referred to as, "2-MIB") is one of the causes of such foul musty odors, and it is limited to levels of 10 ng/L or less in Japanese water quality standards for drinking water. In recent years, concentrations of 2-MIB have risen tremendously in several Japanese rivers due to the odor-producing cyanobacterium *Phormidium autumnale,* which has had an impact on water treatment (Ando et al. 2011; Takeuchi et al. 2015). *P. autumnale* appears as a black crust on riverbeds (Figures 1A and 1B) and has filaments when we observed under a microscope (Figure 1C). Despite the fact that urbanization has not progressed much in the upper basin of the Tama River, Tokyo's high-quality water source, and there were no major changes in water quality in the river over the long term. Nonetheless there were large concentrations of 2-MIB in the water caused by this cyanobactrium (Tsunoda et al. 2014; Oikawa et al. 2015). Since FY2011, large concentrations of 2-MIB have frequently been detected in raw water at the Ozaku Purification Plant (Figure 2), which draws from the upper basin of the Tama River. At the plant, these concentrations hit maximum level in the history of 220 ng/L of raw water at 5:00 PM on August 17, 2013.

A large number of cases have been reported on musty odors in the past; however, most of them have been caused by planktonic cyanobacteria and actinomycetes in eutrophic lakes and marshes (Zaitlin et al. 2003; Kitazawa et al. 2008; Akiba 2008; Yano 2008; Sugiura et al. 2008). Only a few studies have been reported on 2-MIB production by riverbed-attached cyanobacteria in clear rivers (Mori et al. 2009; Hasegawa et al. 2014).

The 2-MIB-affected Ozaku Purification Plant (processing capacity of 280,000 m^3/day) is located in

the western part of the Tokyo Metropolis and draws surface water from the Hamura Intake Weir of the upper Tama River basin. The plant treats water via coagulation-sedimentation and rapid filtration, and it features equipment that introduces powdered activated carbon into the water.

The Ozaku Purification Plant uses sodium hypochlorite as a disinfectant. Prechlorination was used at the plant in the past; however, after powdered activated carbon began to be frequently introduced to remove 2-MIB, a problem in which 2-MIB-attached powdered activated carbon would be exposed to sodium hypochlorite arose. It is commonly known that when this occurs, oxidation causes the attached 2-MIB to be re-released into the water (Edzwald, 2011). Also, it is concerned that this will result in inadequate 2-MIB removal. Thus, it was planned to implement an intermediate chlorination process at the Ozaku Purification Plant; however, there was no space between the sedimentation tank and the rapid filtration tank to install a chlorine-mixing basin. To resolve this problem, a barrier was constructed in the waterway to flow the water from the sedimentation tank to the rapid filtration tank for mixing promotion of sodium hypochlorite and precipitated water. After hydraulic testing at a pilot plant in FY 2014, intermediate chlorination processing began at the Ozaku Purification Plant in July 2016.

In this study, we conducted a field survey in the upper basin of the Tama River to assess 2-MIB concentrations and *P. autumnale* proliferation in water, as well as an indoor culturing experiment to clarify the effect of water temperature on *P. autumnale* proliferation and 2-MIB production, which is still unknown. Additionally, in terms of 2-MIB removal methods, we compared the intermediate chlorination implemented at the Ozaku Purification Plant with prechlorination, from a water quality perspective, and clarified the effects thereof in this paper.

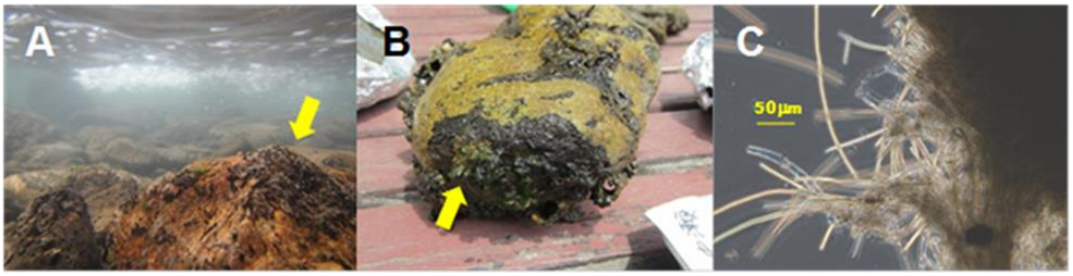

Figure 1. Photos of *P. autumnale* (A: Riverbed, B: Collected rock, C: Enlarged under microscope)

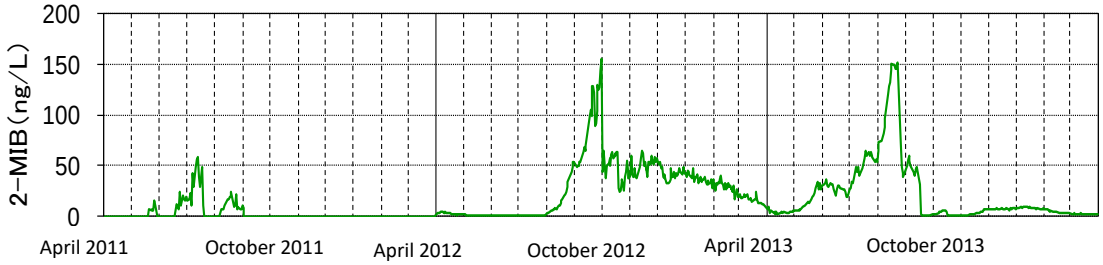

Figure 2. Transition of 2-MIB Concentration in Raw Water at Ozaku Purification Plant (at 9:00 am)

METHODOLOGY

Field survey

Figure 3 shows an overview of the upper Tama River basin and sites at which *P. autumnale* proliferation was surveyed.

From FY 2011 through FY 2013, we investigated several times about 2-MIB concentrations in the waters of the upper Tama River basin within the range of Hamura Intake Weir, which is intake of Ozaku Purification Plant and a point approximately 20 km upstream from the weir. After taking

water samples from the river, we measured 2-MIB concentrations by using a purge and trap-gas chromatography-mass spectrometer (PT-GC-MS) (JWWA, 2011). We plotted 2-MIB concentrations on a graph against distance from Hamura Intake Weir to test where along the river changes in 2-MIB concentrations could be observed.

Additionally, from FY 2011 through FY 2013, we established survey sites at five locations (A to E) towards upstream from Hamura Intake Weir to assess *P. autumnale* proliferation. Points A and B were survey sites from the very beginning; however, Points C D, and E were added in September 2013 and later.

Roughly once per month from FY 2011 through FY 2013, a maximum of three locations per survey site were selected for sampling, and three rocks per location were taken from the riverbed at random. Crusted-on algae were brushed off each rock's surface ,which was 25-cm^2 (5 cm * 5 cm) per rock and suspended it in pure water, and samples were taken in fixed quantities. *P. autumnale* was counted under a microscope by setting 100 μm of filaments as one unit and distinguishing that shape from other types of algae (JWWA, 2011). These results were converted to units per cm^2 of rock's surface. We calculated the average count for all locations at each survey site and plotted these results on a chronological graph to examine seasonal change in *P. autumnale* proliferation.

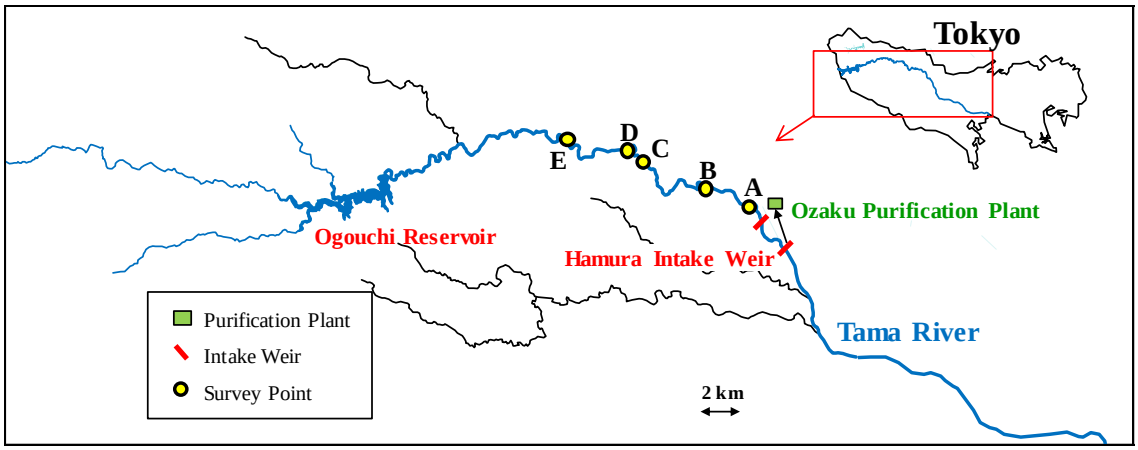

Figure 3. Map of Tama River System and Survey Points of *P. autumnale*

Indoor culturing experiment on cyanobacteria

Indoor culturing experiments were carried out by using isolated *P. autumnale* initially collected from the upper Tama River basin. We used two water temperatures for the experiments, 22 °C and 6 °C, to reflect the summer highs and winter lows in the upper basin. Beside, each temperature was maintained at a constant level throughout the experiments. Under the two temperature conditions, we compared difference in chronological changes by temperature in the *P. autumnale* growth factor, dissolved 2-MIB concentrations external to the algae, and total 2-MIB concentrations including 2-MIB within the algae. Through this comparison, we clarified the effect of water temperature on *P. autumnale* proliferation and 2-MIB production, as well as tested whether inferences based on field surveys can be verified.

Firstly, we precultured isolated *P. autumnale* and cultivated it until we achieved sufficient quantity for the experiment. We prepared a sufficient number of φ18 mm test tubes and added 10 mL each of sterilized CT culture medium (JWWA, 2011). Thereupon, we inoculated precultured *P. autumnale* into those tubes and stored them in incubators (Bioshaker BR-43FL, TAITEC Co.) to the specific temperatures and other settings of the experiment to begin the culturing experiment. Secondly, we used an ultrasonic homogenizer (sonicator; VP-30S, TAITEC Co.) on clumps of precultured *P. autumnale* cyanobacteria to create homogenized culture media, and then added those media to test tubes to achieve concentrations of around 100-200 units/mL and standardize initial cell density across test tubes. Below table 1 shows the specifications of this culturing experiment.

Table 1. Indoor culturing experiments – specifications

	Culturing specifications
Water temperatures	6 ℃and 22 ℃
Culture media	CT culture media 10 mL
Culture containers	φ18 mm test tubes
Illumination	3,000 lux
Light-dark cycle	12 hours of light, 12 hours of dark

As every arbitrary number of days passed, we picked up several test tubes from each of the incubators, and measured *P. autumnale* cell density and 2-MIB concentrations. We measured each item by using the same methodology as in the field surveys. Cell density was measured under a microscope by counting 100 μm of *P. autumnale* filaments as one unit, while 2-MIB concentrations were measured using a purge and trap-gas chromatography-mass spectrometer. For measuring cell density of *P. autumnale*, an ultrasonic homogenizer (sonicator) was used to break up *P. autumnale* adhering to test tube walls and create homogenized culture media for use in measurement. For measuring dissolved 2-MIB concentrations, filtrates were created by running unhomogenized culture media alone through a GF/F filter (particle retention capacity: 0.7 μm, General Electric Co.), and the filtrates were used for measurement. For measuring total 2-MIB concentrations, an ultrasonic homogenizer (sonicator) was used to break up *P. autumnale* adhering to test tube walls and disperse it throughout the culture media. Subsequently, sodium hypochlorite was added and processed for 30 minutes to dissolve 2-MIB inside the cells out into the culture media. Afterward, the media were run through GF/F filters to create filtrates that were used for measuring total 2-MIB concentrations. We measured two or three test tubes for each item and calculated average values. The *P. autumnale* growth factor was calculated by dividing thus measured cell density by the initial cell density.

Testing the 2-MIB removal effectiveness by the intermediate chlorination

At the Ozaku Purification Plant, the amount of 2-MIB (ng/mg) removed per 1 mg of powdered activated carbon was calculated by using the following formula.

$$Q_{MIB} = (C_{RW} - C_{TW}) / A$$

Q_{MIB}: Volume of 2-MIB removed (ng/mg) per 1 mg of powdered activated carbon; C_{RW}: 2-MIB concentration in raw water (ng/L); C_{TW}: 2-MIB concentration in treated water (ng/L); A: Powdered activated carbon dosage (mg/L)

This formula was used to calculate volumes removed both before and after the implementation of the intermediate chlorination. 2-MIB concentrations in raw water and volumes of 2-MIB removed per 1 mg of powdered activated carbon were then plotted on a graph for comparison to test the increase in 2-MIB removal efficiency achieved by the intermediate chlorination. For the pre-implementation period, data were used from the high-temperature seasons of FY 2013, FY 2014, and FY 2015; and for the post-implementation period, data were used from the high-temperature season of FY 2016. From each period, days were extracted on which 15 ng/L or more 2-MIB was detected in raw water, and these days were used for the comparison. For 2-MIB concentrations in treated water (C_{TW}), the average value was used for each period.

RESULS and DISCUSSION

Field survey

Below Figure 4 shows survey results for 2-MIB concentrations in river water in the summer period. For FY 2011 and FY 2012, no 2-MIB was detected at the site located around 15 km upstream of Hamura Intake Weir. However, 2-MIB concentrations rose the further as they flowed into downstream of Tama River, and spiked tremendously at the downstream of a site, which was

located around 5 km upstream of Hamura Intake Weir. Especially in FY 2012, there is a clear numerical increase in proliferation volume of *P. autumnale* at Point A (around 4 km upstream of Hamura Intake Weir). Meanwhile, FY 2013 differs from the preceding two years. There was a drastic spike in 2-MIB concentrations in the section running between locations approximately 10 km and 15 km upstream of Hamura Intake Weir. The winter period exhibited no sudden increases in 2-MIB concentrations compared to the summer period, throughout the entire surveyed area.

Below Figure 5 shows survey results for *P. autumnale* proliferation volume. Of these, Points C, D, and E were newly added as survey sites since September 2013 due to the sharp increases in 2-MIB concentrations in locations approximately 10 km and 15 km upstream of Hamura Intake Weir.

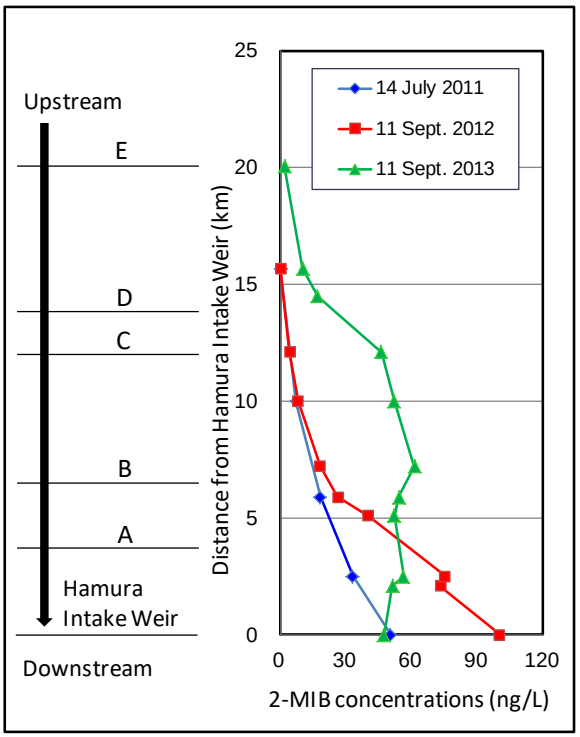

Figure 4. Survey results, 2-MIB concentrations in river water

In FY 2012, high volumes of *P. autumnale* were detected intermittently at Point A (approx. 4 km upstream of Hamura Intake Weir), the vicinity of which had also exhibited marked increases in 2-MIB concentrations in river water, and *P. autumnale* had proliferated at Point A in the winter period. Located approximately 7 km upstream of Hamura Intake Weir, Point B demonstrated trends similar to Point A, and in the winter of February 2013, an unprecedentedly large 264,000 unit/cm^2 concentration of *P. autumnale* was detected at Point B. In fact, it has been reported that clumps of cyanobacteria created by *P. autumnale* were observed in a river in New Zealand with water temperatures of 8 ℃ (Heath & Wood, 2010). Although cyanobacteria has been considered as proliferate in the high water temperatures of summers rather than the low temperatures of winter, the results of the current study led to the conjecture that *P. autumnale* can adapt to the low water temperatures in winter to proliferate.

However, across the entire year-long period, proliferation volumes of *P. autumnale* did not necessarily align with trends in 2-MIB concentrations in raw water at Ozaku Purification Plant. When 2-MIB concentrations in raw water were extremely high in Ozaku Purification Plant in September 2012 (Figure 2), proliferation volumes of *P. autumnale* at Point A and Point B were not especially high, reaching only 18,000 unit/cm^2 and 3,000 unit/cm^2 respectively (Figure 5). Moreover, after 2-MIB concentrations in raw water at Ozaku Purification Plant fell to 50 ng/L due to flooding caused by rains in early October 2012, concentration levels did not rise again but rather gradually dropped off (Figure 2). Yet at Points A and B, *P. autumnale* continued to proliferate after October 2012, each one reached its maximum in February 2013 (Figure 5).

Proliferation volume of *P. autumnale* in this period reached around 7 times more volumes than the one in September 2012 (summer) at Point A, and around 90 times at Point B. In other words, *P. autumnale* may be less numerous in the high-temperature in summer period than the low-temperature period, 2-MIB concentrations in raw water grow. Meanwhile, even though *P. autumnale* proliferates in the low-temperature winter period, 2-MIB concentration in raw water does not increase that much. The cause of this is conjectured to be that 2-MIB produced and emitted by these cyanobacteria is affected by water temperatures.

Additionally, *P. autumnale* proliferated continuously by tens of thousands of units/cm^2 at Points C and D since September 2013; however, at Points A and B, *P. autumnale* volumes were decreased

relative to before. Through this, it was confirmed that *P. autumnale* proliferation sites on rivers could change. Finally, Point E exhibited only a little *P. autumnale* proliferation.

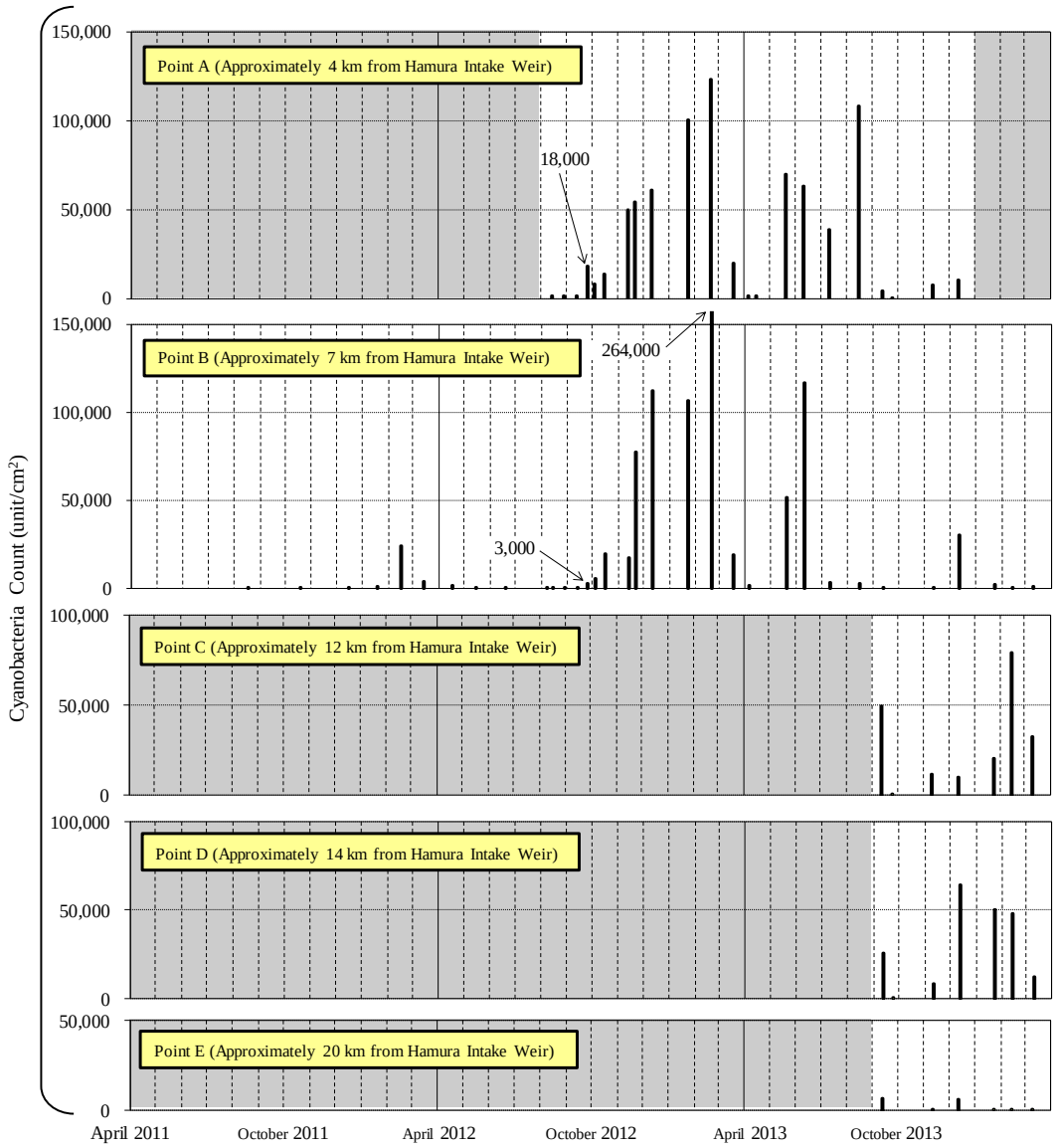

Figure 5. Proliferation volumes of *P. autumnale* (filled sections indicate that no survey took place)

Cyanobacteria indoor culturing experiments

Below 6A shows changes in the growth factor of *P. autumnale* at water temperatures of 22 °C and 6 °C. At a water temperature of 22 °C, *P. autumnale* enters a logarithmic growth phase (in which cells divide frequently and increase exponentially) until the 7th day after it begins culturing, followed by a stationary phase (in which cell multiplication and death are in equilibrium) until the 21st day. The growth factor of *P. autumnale* in the stationary phase was around 400 times at a water temperature of 22°C. Meanwhile, at a water temperature of 6 °C, *P. autumnale* proliferation speeds were considerably lower than at 22 °C; however, the two reached equal growth factors on the 42nd day after culturing began. These results show that even at low water temperatures, *P. autumnale* can multiply to reach the same cell density as in high-temperature water over the long term. In field surveys, as well, it was confirmed that high quantities of *P. autumnale* were proliferating in actual rivers in the winter period in 2012. Indoor culturing experiments provided support for these results. It was inferred that since the upper Tama River basin experiences almost no flooding in the winter in the typical year, *P. autumnale* is able to continue proliferating on the riverbed without peeling off, even if the speed of proliferation is low.

Below Figures 6B and 6C show changes in dissolved 2-MIB concentrations and total 2-MIB

concentrations at water temperatures of 22°C and 6°C.. Dissolved 2-MIB concentrations and total 2-MIB concentrations increased as days passed at both temperatures; however, the speed of growth at 6 °C was quite lower than at 22 °C. 2-MIB production and emission was found to be accelerated at the higher water temperature. Nonetheless, it was considerably suppressed at the lower temperature. In field surveys, *P. autumnale* was present in smaller quantities at high water temperature periods in the summer season than at low water temperature periods; however, 2-MIB concentrations in raw water were higher for higher temperatures. Meanwhile, it was found that *P. autumnale* proliferated in low water temperature periods in the winter season; in contrast, that 2-MIB concentrations were not especially high in raw water. These results support our findings and we verified the fact that 2-MIB production and emission by cyanobacteria are affected by water temperature through the indoor culturing experiments.

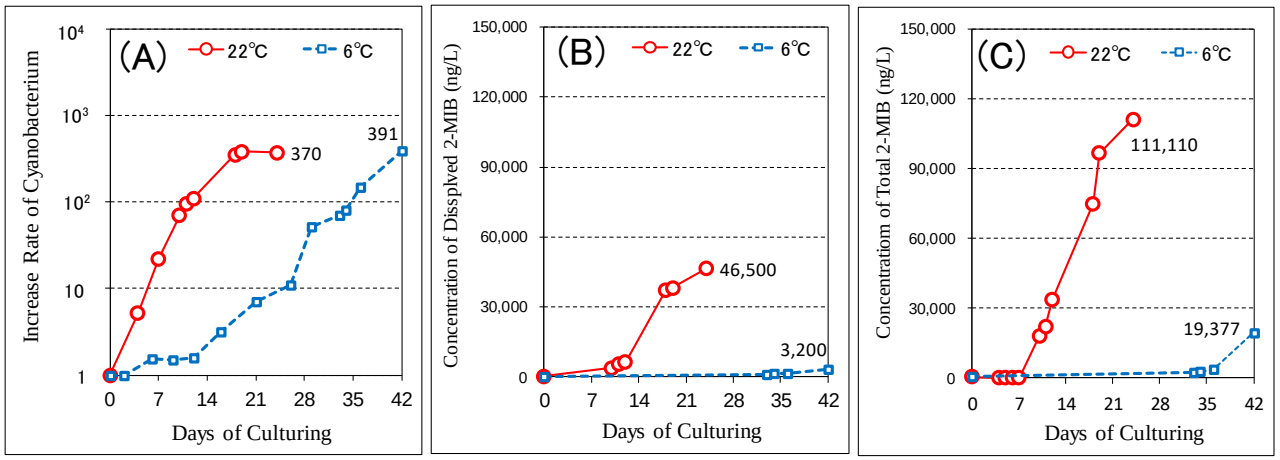

Figure 6. Change in Increase Rate of *P. autumnale*, Concentration of 2-MIB in a Dissolved Form and Concentration of Total 2-MIB at High and Low Water Temperatures

Testing the 2-MIB removal effectiveness by the intermediate chlorination

Below figure 7 shows the amount of 2-MIB removed per 1 mg of powdered activated carbon by prechlorination and the intermediate chlorination, for before and after the implementation of the intermediate chlorination.

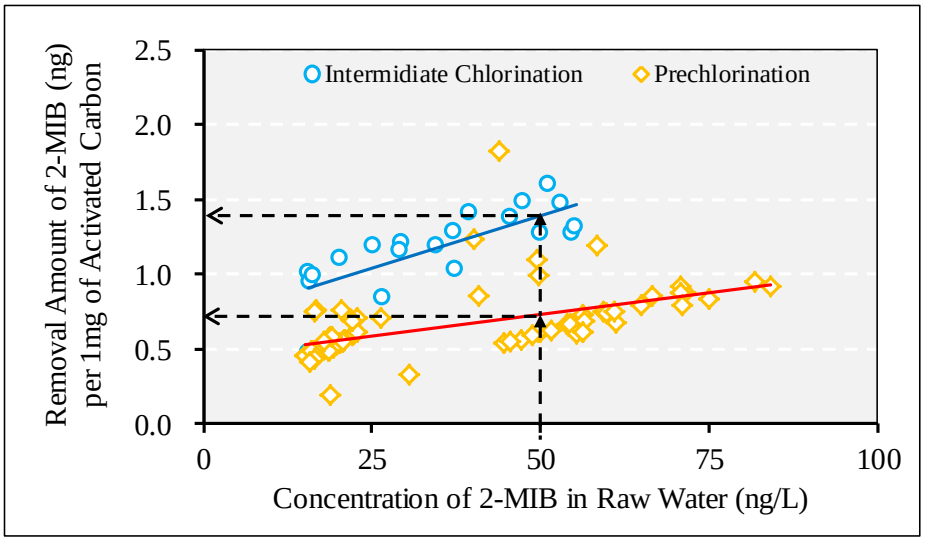

Figure 7. Quantities of 2-MIB removal per 1 mg of powdered activated carbon (A comparison of pre- and post-implementation of intermediary chlorination)

Comparing prechlorination data (n=64) with intermediate chlorination data (n=20), intermediate chlorination clearly removed more 2-MIB per 1 mg of powdered activated carbon overall. Given a 2-MIB concentration of 50 ng/L in raw water, the amount of 2-MIB removed per 1 mg of powdered

activated carbon was around 0.7 ng for the prechlorination method and improved to around 1.4 ng for the intermediate chlorination, according to the straight-line approximation in the graph below. This indicates that the introduction of the intermediate chlorination method approximately doubled 2-MIB removal efficiency at the Ozaku Purification Plant.

CONCLUSION

Through field surveys, we clarified the relationship between *P. autumnale* proliferating in a natural environment and 2-MIB concentrations in raw water at purification plants. We also established the hypothesis that 2-MIB production and emission by these cyanobacteria are affected by water temperatures. Through indoor culturing experiments, we tested that hypothesis and clarified the characteristics of *P. autumnale* proliferation and 2-MIB production with respect to water temperature.

At purification plants which were affected by 2-MIB, the Tokyo Metropolitan Government Bureau of Waterworks conducted hydraulics experiments in order to improve purification facilities. This paper succeeded in quantitatively assessing the outcomes of those improvement efforts from the perspective of water quality by drawing on water quality data.

In conclusion, this study proves that *P. autumnale* could adapt to low water temperatures in the winter period and continue to proliferate even though 2-MIB production was suppressed. In addition, this illustrates the need for water utilities to be mindful of musty odors in the winter. As aforementioned, 2-MIB production by *P. autumnale* has been reported by multiple water utilities throughout Japan, and we hope that this paper will be helpful for water quality management hereafter.

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