EFFECTS OF TEMPERATURE AND ALGAE BIOMASS ON ALGAE DECAY AND THE PRODUCTION OF DIMETHYL TRISULFIDE

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(Received 25th Jun 2023; accepted 22nd Sep 2023)

Abstract. The rise of temperature and the massive accumulation of cyanobacteria are considered to be the two main factors causing the decomposition of algae and leading to black and odorous water. In this test, the characteristics of algae decay and the production of typical odorous organics under different conditions were studied by setting different temperatures and configuring cyanobacteria solutions with different initial densities. The results proved that the increase of temperature and initial density significantly promoted the decomposition of algae. When the ambient temperature reached 33℃ and the initial algae density reached 1.0×10^9 cells/L, more than 60% of the algae biomass would decrease on the third day of the test. When the average algae density of the configured water was 1.0×10^8 cells/L (the stacking thickness was 1.2-1.4 cm on the surface of the water) and the temperature was 33℃, the maximum concentration of dimethyl trisulfide (DMTS), the typical odorant, was up to 8614.5 ng/L, while the maximum concentration was 448.4 ng/L on the temperature of 25℃. Temperature has a great influence on the production of odor substances and the increase of temperature promotes the formation of sulphorous compounds.
Keywords: *cyanobacteria, algae density, temperature, algae decay, odorant*

Introduction

Large scale algae blooms have been considered as one of the main reasons for the occurrence of black and odorous water in lakes (Guo, 2007; Feng et al., 2014; Huisman et al., 2018; Watson et al., 2016). The decomposition of large-scale algae will cause the production of odorants or black blooms (Duan et al., 2014; Zhang et al., 2010). There are many factors affecting algae outbreak, including physical, chemical, biological and other aspects. Among these factors, temperature is considered to be the most direct and significant parameter affecting the growth and decomposition of planktonic algae (Kingsolver, 2009; Ibelings et al., 2011), and the production of odor matters (Yin and Wu, 2016; Yu et al., 2016). Several studies have reported variations in the growth rate and odor matters production under different temperatures, but inconsistent conclusions were obtained for different organisms. When the temperature increased from 15℃ to 30℃, the specific growth rate of three filamentous algae would increase from 0.12 d^{-1} to 0.70 d^{-1} (Gao et al., 2020). *Cylindrospermopsis raciborskii* is a kind of freshwater cyanobacteria and the optimum temperature for its growth is 30℃, not 25℃ or 35℃ (Nie et al., 2016). The lowest growth rate of *Pseudanabaena sp.* and the maximum 2-methylisoborneol production were observed at 35℃. The lowest growth rate of *Anabaena ucrainica* and the highest geosmin

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2106_55955605

production were observed at 10℃, compared with that at 25℃ and 35℃ (Wang and Li, 2015). However, the investigation of Zhang (Zhang et al., 2010) found that the highest concentration of geosmin appeared in July, while the highest concentration of 2-methylisoborneol appeared in April. Obviously, the temperature of Taihu in July was higher than that in April. β- Cyclocitral of the particle state peaked in May, July and September, β- Ionone of the particle state had a high concentration from July to October, on site in the North of Gonghu Bay in Taihu Lake (Ma et al., 2013). However, the maximum β- Cyclocitral and β- Ionone release concentrations were observed at 35°C compared with that at 25°C and 30°C (Huang et al., 2018). These studies indicated that the change of temperature has an impact not only on the growth and decay of algae, but also on the production of odor substances in the process of algae decay. However, there is still a lack of quantitative research on the correlation between temperature and algae decay. The thioether odorants were considered the main substances (Duval and Ludlam, 2001; Shen et al., 2014) in black and odorous water in the process of algae decay. Similarly, there is also a lack of research between temperature and the production of these substances. In addition, the odorous matter may come from the decomposition of cyanobacteria (Yu et al., 2016; Lanciotti et al., 2003; Smith et al., 2008), so the concentrations of odorous substances were affected by the cyanobacteria biomass. However, there is also a lack of quantitative research on the response relationship between algae biomass and algae decomposition and the production of odorants.

In this paper, the following two parts will be studied. In the first part, according to the meteorological characteristics (Wang et al., 2011) and the surface stacking density of cyanobacteria during the occurrence of odorous black water agglomerate, algae aqueous solutions with different initial algae density were prepared respectively, and the influence of temperature on the decay characteristics of cyanobacteria under different initial conditions was analyzed. In the second part, the influence of temperature and the initial algae biomass on typical thioether odorants were analyzed. These experiments would quantitatively analyze the effects of temperature changes and blue-green algae accumulation on algae decomposition and odor producing substances. The findings would provide theoretical support for the prevention and control of odorous black water agglomerate, and these factors would serve as warning indicators for the occurrence of black and odorous water.

Materials and methods

Test materials and devices

The materials used in this test mainly include cyanobacteria, Taihu raw water and ultra-pure water, of which cyanobacteria and Taihu raw water were collected in local areas with high incidence of odorous black water agglomerate. The sampling point was close to the Shatang Port in the Zhushan Bay of Taihu (31°24’44.2”N, 120°01’8.1”E). Fresh cyanobacteria were collected on the surface of the Taihu water using a plankton net (PTN-25), which the aperture was 0.064 mm. Through microscopic examination, more than 95% of them were *Microcystis aeruginosa*. Taihu raw water was collected in situ. After being collected, the water was used for test after removing large particle suspended solids and plankton through a screen.

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This test included two parts. In Part I, cyanobacteria and ultra-pure water were used to prepare algae aqueous solutions according to *Table 1,* and then placed in a 500 ml culture flask. Each test group was set with 2 parallel.

Table 1. *Part I test setup*

		Initial algae density (cells/L)	
1 (25℃)	1.00×10^{10}	1.00×10^{9}	1.00×10^{8}
2 (30℃)	1.00×10^{10}	1.00×10^{9}	1.00×10^{8}
3 (33℃)	1.00×10^{10}	1.00×10^{9}	1.00×10^{8}

In part II, the test device included three plexiglass columns with a diameter of 0.25 m and a height of 1.00 m. Cyanobacteria and Taihu raw water were used to prepare algae aqueous solution. In the first group of tests, three identical algae solutions with the initial cyanobacteria density 1.00×10^{8} cells/L were placed at 25℃, 30℃ and 33℃ respectively under static, constant temperature and shading conditions. In the second group of tests, three algae solutions with the initial cyanobacteria density 1.00×10^{6} cells/L, 1.00×10^{7} cells/L, and 1.00×10^{8} cells/L were placed at 30℃ under static, constant temperature and shading conditions.

Sampling and analytical method

Part I of the experiment began when the density of cyanobacteria began to decline. Samples were taken every other day for a total of 17 days. Part II of the experiment started from the preparation of algae solutions until DMTS could not be measured, a total of 14 days.

The algae count in part I was performed by the blood cell counting plate method (MEP, 2002). Count under the olympus-bx41 microscope at 400 or 1000 times, shake the water sample well before sampling and testing, and test the same water sample in parallel for 3 times. In part II the odorant detection was detected by a purging and trapping (P&T) pretreatment coupled with the gas chromatographic mass spectrometry (GC-MS, Thermo Scientific ITQ 1100TM) according to previous studies (Wang et al., 2014). Quantitative analysis was conducted in selective ion scanning mode (SIM).

Data processing

Algae decay ratio was calculated using *Equation 1,* where y_1 represented the algae biomass at a certain point in time (cells/L); y_2 represented the initial algae biomass (cells/L).

$$p = \frac{y_2 - y_1}{y_2} \quad \text{(Eq.1)}$$

Algae decay rate was calculated using *Equation 2,* where p_1 and p_2 represented algae decay ratio at the beginning and the end of a time interval, respectively; and (t_2-t_1) represented a certain time interval (d).

$$x = \frac{p_2 - p_1}{t_2 - t_1} \quad \text{(Eq.2)}$$

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Test results and discussion

Characteristics of algae decomposition at different ambient temperatures

Figure 1 showed the algae decomposition characteristics at different temperatures (25℃, 30℃, 33℃) when the initial cyanobacteria density was 1.0×10^{10} cells/L, 1.0×10^{9} cells/L, 1.0×10^{8} cells/L, respectively. When the initial density was 1.0×10^{10} cells/L (*Fig. 1a*), the algae decayed fastest at 33℃, and the decay ratio reached about 80% on the third day. At this time, the algae biomass in the groups at 30℃ and 25℃ decreased by 50% and 17%, respectively. Until the 11th day, the algae biomass at 30℃ decreased by 84%, and at 25℃ decreased by 80% on the 13th day. When the initial density was 1.0×10^{9} cells/L (*Fig. 1b*), the algae decayed fastest at 33℃ similarly, and the algae decay ratio was 61% on the third day. At this time, the algae biomass in the groups at 30℃ and 25℃ decreased by 44% and 22% respectively. Until the fifth day, the algae biomass at 30℃ decreased by 61%. Until the ninth day, the algae biomass at 25℃ decreased by 62%. When the initial density was 1.0×10^{8} cells/L (*Fig. 1c*), the decay ratio of cyanobacteria in each test group was between 10% and 30% on the third day. The difference among the groups was not obvious in the entire test period.

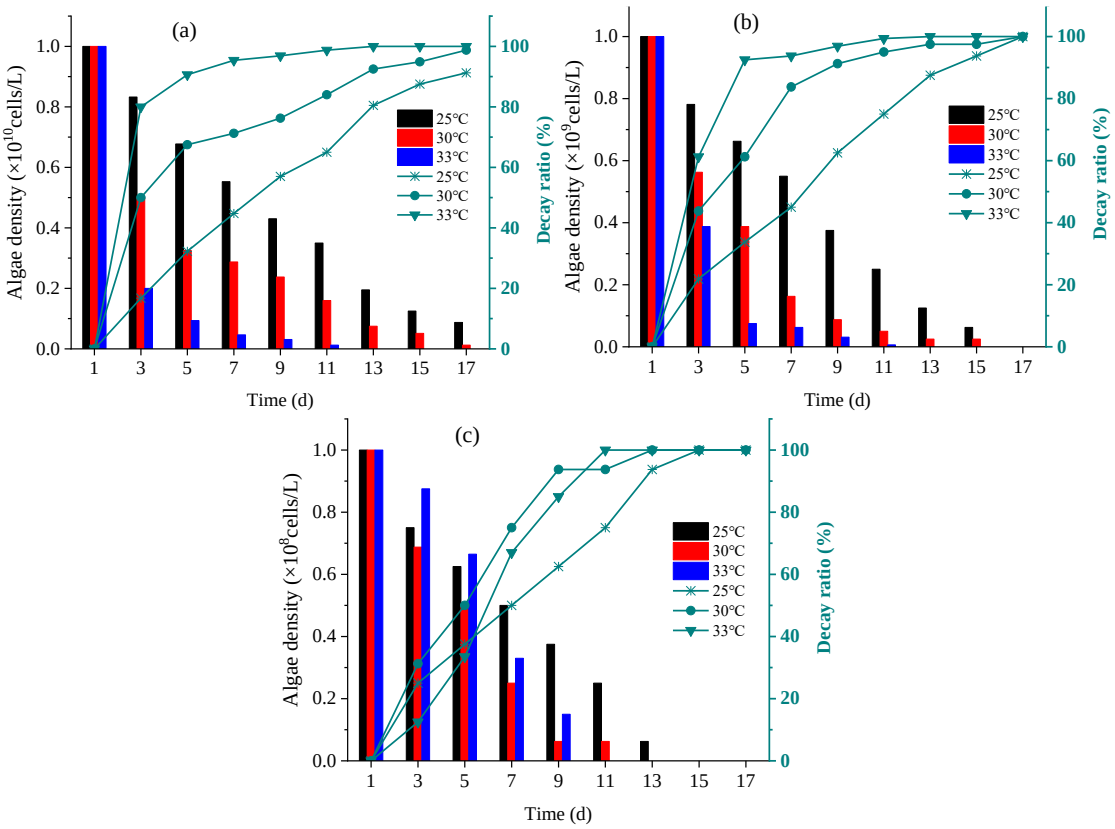

Figure 1. *Algae decomposition characteristics with an initial density of 1.0×10^{10} cells/L (a), 1.0×10^{9} cells/L (b) and 1.0×10^{8} cells/L (c) at different temperatures*

It can be seen that the initial algae density was 1.0×10^{10} cells/L or 1.0×10^{9} cells/L, the decay ratio of the group with higher temperature was higher than that of the group with lower temperature during the whole test period. The increase of temperature

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greatly promoted the decomposition of algae. The higher the initial algae density is, the greater the influence of temperature on algae decomposition is.

As shown in *Figure 2*, the initial accumulated algae density was 1.0×10^{10} cells/L (*Fig. 2a*) and 1.0×10^{9} cells/L (*Fig. 2b*), the decay rate trend of algae at 33℃ and 30℃ was almost consistent. The decay rate was high in the early stage of the test (3-5 days), and it decreased and tended to be even in the middle and late stages of the test. This may be because in the early stage of the test, the algae with high bulk density competed fiercely in the case of extreme lack of nutrients, leading to the rapid decay of algae. In the middle and late stages, due to the reduction of the absolute number of living algae and the early decomposition of algae, nitrogen and phosphorus nutrients were released into the environment, making the remaining algae obtain certain nutrients to grow and multiplication, thus reducing the decay rate of algae. Although the decay rate trend was consistent, the groups with high temperature were significantly higher than those with low temperature with the same high algae density at the beginning of the test, indicating that high temperature would significantly promote the decay of algae. The higher the temperature, the higher the decay rate.

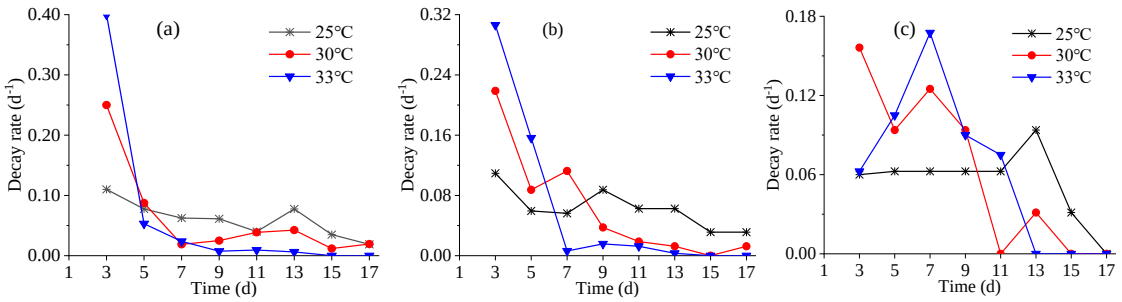

Figure 2. *Algae decay rate with an initial density of 1.0×10^{10} cells/L (a), 1.0×10^{9} cells/L (b) and 1.0×10^{8} cells/L (c) at different temperatures*

When the initial algae density was 1.0×10^{8} cells/L (*Fig. 2c*), the algae decay rate trend at 33℃ and 30℃ was also large at the initial stage of the test and decreased at the middle and late stages of the test, but the decay rate at the initial stage was similar, which showed that when the density of algae was low, the effect of temperature on the decay of algae was not obvious. Compared with high temperature, the decay rate of algae with different algae densities was alike at 25℃, and the fluctuation amplitude is small.

Characteristics of algae decomposition with different initial algae accumulation densities

Figure 3 showed the decomposition characteristics of algae with different initial densities at the same temperature. As shown in *Figure 3a*, at 33℃, the biomass of high-density (1.0×10^{10} cells/L), medium-density (1.0×10^{9} cells/L) and low-density (1.0×10^{8} cells/L) algae decreased by about 80%, 60% and 12% respectively on the third day. At the same high temperature, the decay rate of high-density algae was the fastest, which was significantly higher than that of medium-density algae, and much higher than that of low-density algae. At 30℃ (*Fig. 3b*), the decay ratio of high-density and medium-density algae were similar in the first 5 days, reaching about 60% in the fifth day, more

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than that of the low-density, which reached about 50%. At 25℃ (*Fig. 3c*), there was no significant difference in decay ratio among groups with different initial densities.

It can be seen that the higher the temperature is, the greater the difference of algae decay between different initial algae densities is, and the higher the algae density is, the faster the decomposition is.

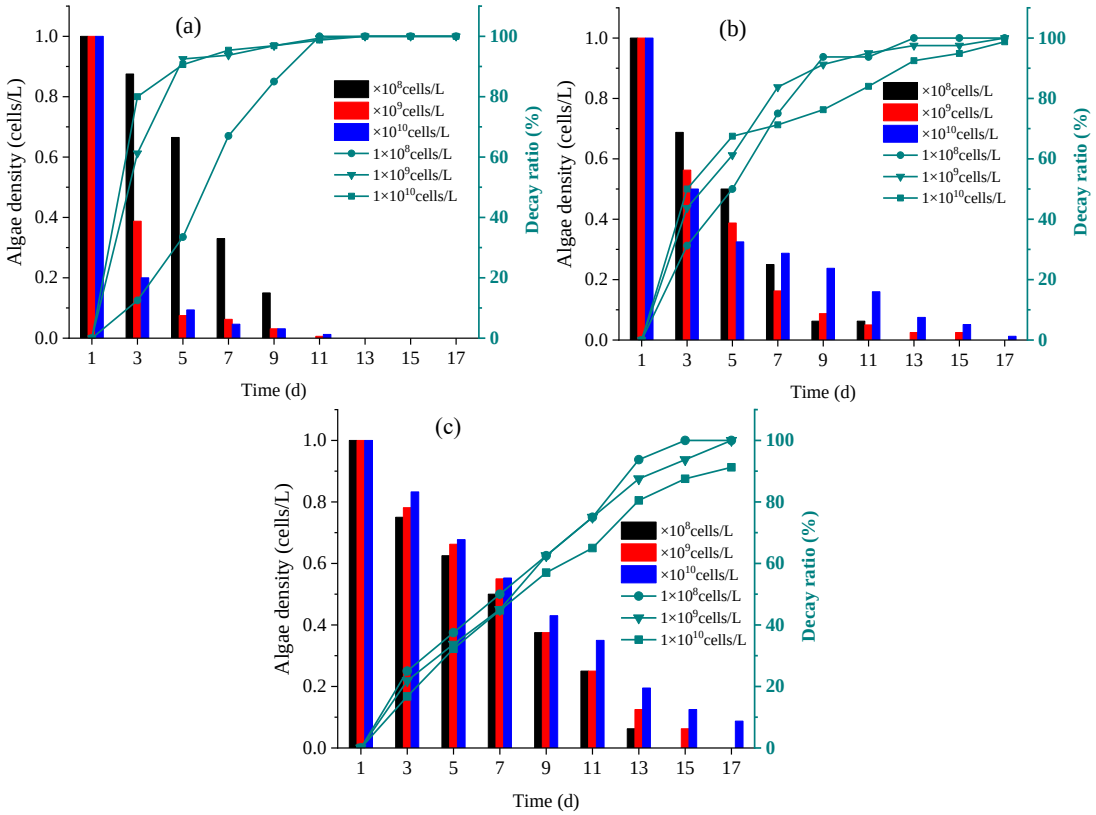

Figure 3. *Algae decomposition characteristics with different initial densities at 33℃ (a), 30℃ (b) and 25℃ (c)*

As shown in *Figure 4a* and *b*, at the temperature of 33℃ and 30℃, the decay rate trend of high-density and medium-density was almost consistent, which was large at the beginning of the test and much higher than that of low-density group. In the middle and later period of the test, the decay rate decreased and tended to be even. At 25℃ (*Fig. 4*c), the decay rate of each group was low throughout the test period, which was below $0.12d^{-1}$, and the fluctuation amplitude was small.

According to the above analysis, under high temperature conditions, the decay rate of algae with high-density and medium-density were both high. On the third day, about 60% - 80% of the algae biomass decreased, while the decay rate of algae with low-density was obviously low and until the 7th day, about 80% of biomass decreased. Under low temperature conditions, the decay rate of algae with the three initial densities were all low during most of the test period, and so the biomass decreased about 50% on the 7th day. It can be seen that higher temperature and algae accumulation with high densities have promoted the decay of algae, while at 25℃ or lower, the initial density is lower than 1.0×10^8 cells/L, the decay rate of algae is low, which is not conducive to the sinking and decay of algae.

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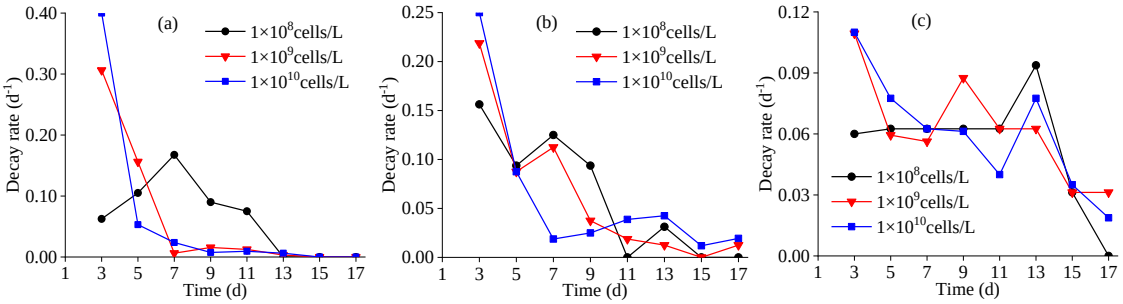

Figure 4. *Algae decay rate with different initial densities at 33℃ (a), 30℃ (b) and 25℃ (c)*

Production and variation of the typical odorant under different conditions

DMTS is considered to be one of the typical odorants of algae induced local black and odorous water, and the odor threshold is 10 ng/L (Deng et al., 2011). *Figure 5* showed the variation of DMTS in the test water with an initial algae density of 1.0×10^8 cells/L at different temperatures. In this test, when the average algae density of the configured water was 1.0×10^8 cells/L, due to the floating of algae, the stacking thickness on the surface of water was 1.2-1.4 cm and the stacking algae density reached 1.0×10^{10} cells/L. When the temperature was 33℃, 30℃ and 25℃, the maximum concentration of DMTS was 8614.5 ng/L, 7902.9 ng/L and 448.4 ng/L, which was 861 times, 790 times and 45 times of its olfactory threshold, respectively. It can be seen that a large number of odorous organic compounds, DMTS were produced in each water at different temperatures, and the higher the temperature was, the higher the peak concentration of DMTS detected in the water was. In terms of the time of peak, the concentration of DMTS in water at 33℃ reached the peak on the 6th day, two days earlier than the water at 30℃.

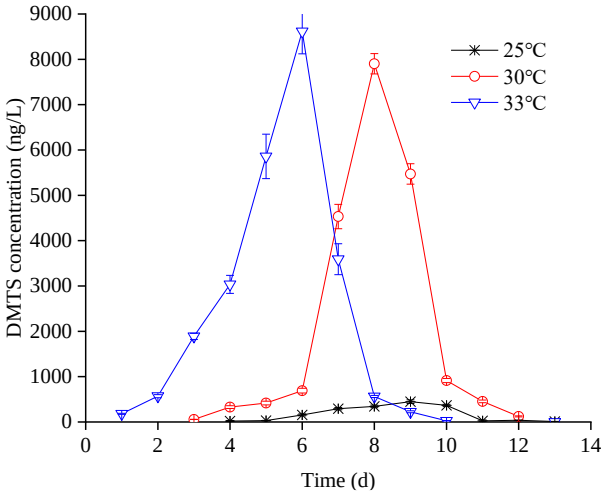

Figure 5. *Variations of DMTS concentrations with an initial average density of 1.0×10^8 cells/L at different temperatures*

The study of temperature on the decay rate of algae showed that when the temperature reached 33℃, 80% of the algae biomass would decrease on the third day of the test. It could be speculated that the high ambient temperature promoted the decay

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DOI: http://dx.doi.org/10.15666/aeer/2106_55955605

and decomposition of algae, making the typical odorous organic DMTS from the algae decay and decomposition rapidly generate in the early stage of the test and reach the maximum value on the 6th day. This may be because temperature can stimulate algae activity and some biochemical processes (Du and Parker, 2012). Similar conclusions have been drawn in other fields (Du and Parker, 2012; Devai and Delaune, 1995). However, at 25℃, the decay rate of algae was slow. DMTS was an intermediate product of algae decomposition (Zinder et al., 1977; Krasner et al., 1985), which would decompose at the same time when it was generated. Therefore, DMTS did not accumulate at 25℃ significantly. During the test, the peak concentration of DMTS was low, 45 times the odor threshold.

Figure 6 showed the production of DMTS in different test waters with different initial algae densities. When the algae density was 1.0×10^6 cells/L, DMTS was detected only from the 6th to 11th days and the maximum was 33.9 ng/L. When the algae density was 1.0×10^7 cells/L, the DMTS concentration reached the maximum value of 891.8 ng/L on the 8th day. When the algae density was 1.0×10^8 cells/L, the DMTS concentration also reached the maximum value of 7902.9 ng/L on the 8th day. At the same high temperature, the higher the algae density, the faster the algae decay (*Figs. 3* and *4*). With the increase of algae density, the concentration of DMTS increased. Thioether substance were considered to originate from the decomposition of sulfur-contained compounds such as methionine (Franzmann et al., 2001; Lu et al., 2013; Sun et al., 2015). In our test, the only apparent difference among groups was the initial algae density. So, it was suggested that algae provided a material basis for the production of odorants. However, the highest concentration of DMTS was not entirely proportional to the algae density. In this test, algae were the only source of odorants. It can be seen that algae accumulation has promoted the production of odorants.

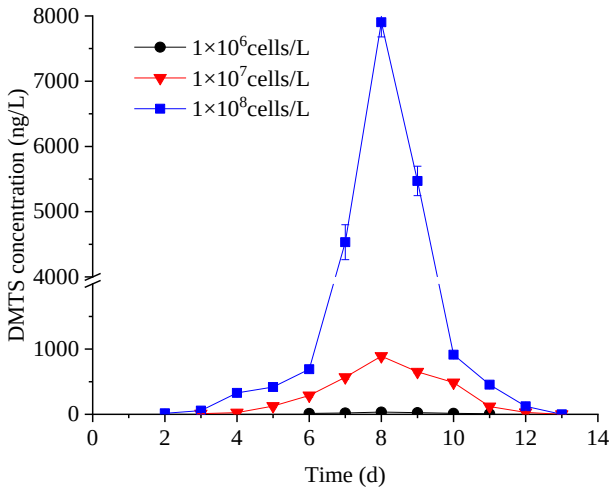

Figure 6. *Variations of DMTS concentrations with different algae densities*

Conclusion

Based on the results and discussions presented above, the conclusions are obtained as below:

(1) Temperature and initial algae biomass have significant effects on algae decomposition. The accumulation of high-density algae and the rise of temperature will aggravate the decomposition of algae. When the ambient temperature reached 33℃ and

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the initial algae density reached 1.0×10^9 cells/L, more than 60% of the algae biomass would decrease on the third day of the test, and the decay rate reaches 0.32 d^{-1}.

(2) Temperature has a significant effect on the production of DMTS. With the same initial algae density, the higher the temperature, the higher the DMTS concentration, and the earlier the peak occurs. When the average initial algae density was 1.0×10^8 cells/L, the peak concentration of DMTS at 33℃ was 19.2 times of 25℃ and 1.1 times of 30℃ respectively. Algae provide a material basis for the production of odorants. With the increase of algae density, the concentration of DMTS increased.

Acknowledgements. This work was financially supported by the Jiaxing Science and Technology Project (2018AY11014) and the Major National Science and Technology Programs on Water Pollution Control and Treatment (2009ZX07101-011).

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