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ORIGINAL ARTICLE

Analysis on the formation condition of the algae-induced odorous black water agglomerate

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Oxidation–reduction potential (ORP);
Dimethyl trisulfide;
Heavy metal-sulfide

Abstract The algae-induced odorous black water agglomerate (OBWA) is a phenomenon in which water turns black and emits odorous gas. It is an ecological and environmental problem that has occurred several times in Taihu, a large eutrophic shallow lake in China. In this study, the collected eutrophic water with different algae densities was used to simulate OBWA. The results revealed that the massive accumulation and death of algae was the substrate source for OBWA. When the algae density reached 1.0×10^8 cells/L in the static and dark condition, at a constant high temperature (30 ± 2 °C), OBWA happened. There was a time difference between the water stinking and blackening with the stinking first. When the oxidation–reduction potential (ORP) value was between −250 and −50 mV, Dimethyl trisulfide (DMTS), the main contributor to the water stinking at the initial stage, and other odorous organics were produced. Water blackening was closely related to the increases of sulfide and dissolved Fe^{2+} concentration. When the ORP value was between −350 and −300 mV, heavy metal containing sulfides such as FeS formed. Therefore, the condition when the water ORP value decreased to about −300 mV was considered the precursor for OBWA formation.

1. Introduction

Lake Taihu (referred to as "Taihu" below) is one of the five largest freshwater lakes in China. It is a typical large shallow lake with an area of 2338 km^2 and an average depth of 1.9 m (Qin et al., 2007). In recent years, large-scale cyanobacteria blooms frequently occurred as a result of eutrophication (Duan et al., 2009; Guo, 2007). What is worse is the odorous black water agglomerate (OBWA), which is induced by cyanobacteria bloom is a serious ecological and environmental problem. It happened frequently from 2007 to 2011 in Meiliang, Gonghu, Zhushan and other bays of Taihu. The water body became black and emitted odorous gas, and the dissolved

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oxygen (DO) concentration reduced to approximately 0 mg/L, which caused the death of a large number of aquatic organisms. It had a severe effect on not only the industrial and agricultural production but also the domestic water quality of the surrounding cities and contributes to ''water crisis''.

The occurrence of OBWA increased the severity of the eutrophication and drew much concern on its impact on water environment (Yang et al., 2008). There have been several studies on the OBWA. Lu et al tracked the OBWA from 2008 to 2009 in the near shore waters of Taihu in Yixing, and analyzed the apparent features of the OBWA (Lu and Ma, 2009). Zhang et al analyzed the water quality data when OBWA occurred in the end of May 2007, and pointed out that the combined effects of nutrient enrichment and industrial pollution led to the occurrence of the OBWA (Zhang et al., 2010). These findings on the formation of OBWA were from indirect observation and analysis, as they were based on the field data investigation during the OBWA period. Liu et al. and Shen et al conducted the researches on the environmental effects of OBWA (Liu et al., 2009; Shen et al., 2011). The occurrence and characteristics of the OBWA induced by the decomposition of the submerged plant *Potamogeton crispus* were studied (Shen et al., 2014). Some studies indicated that the physical and chemical characteristics (e.g. depletion of DO) in the surface sediments were important for the formation of OBWA (Shen et al., 2013). However, these researchers did not conduct sufficient quantitative research on the relationship between the occurrence of OBWA and the large-scale cyanobacteria bloom outbreak or the cyanobacteria concentration. Although many odor episodes were reported to occur in Lake Kasumigaura of Japan (Sugiura et al., 1998; Sugiura and Nakano, 2000), in Lakes of Ontario and New Hampshire (Nicholls et al., 1982), in Lake Dianchi of China (Li et al., 2007), etc., they definitely did not couple with the black water. Meanwhile, the causes of these odorous water bodies, the types of odorous gases and the properties of these water bodies are rather different compared to the situation in Taihu.

In this study, eutrophic water with different algae densities was used to simulate OBWA. The responsive relationships between the aquatic environment and the formation of water stinking and blackening were analyzed. The typical substrates that induced odorous black water agglomerate were identified and the formation mechanisms were further analyzed. These analyses provided theoretical support for the early warning of OBWA and contributed to the construction of a prevention system.

2. Materials and methods

2.1. Test materials and devices

Taihu is located in the downstream region of the Yangtze River (between 30°55′40″–31°32′58″N and 119°52′32″–120°36′10″E). Zhushan Bay is one of the three bays north of Taihu, where OBWA occurred from 2008 to 2011. The sampling point in this study was close to the Shatang Port in the Zhushan Bay of Taihu (shown in Fig. 1, 31°24′44.2″N, 120°01′8.1″E).

The test algae were collected by a PTN-25 plankton net (mesh size of 0.064 mm) and more than 95% were *Microcystis aeruginosa*. The water samples were collected from the surface layer and stored in a polyethylene bucket after the large particles of suspended solids and plankton were removed with the net. The test sediment with a depth of approximately 0.25 m was collected by a gravity sediment collector from the surface layer of Taihu. The collected specimens were preserved well and brought back to the laboratory for subsequent simulative research.

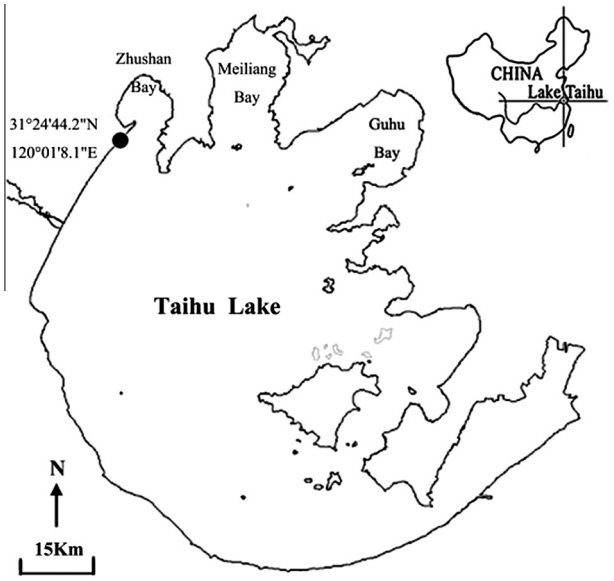

Figure 1 Location of the sampling site in Zhushan Bay, Lake Taihu, China.

Three tests with the algae densities of 1.0×10^6, 1.0×10^7 and 1.0×10^8 cells/L were conducted.

A Plexiglas column with a height of 1.50 m and a diameter of 0.25 m was used as the experimental vessel. Three identical columns (as triplicates) for each treatment were used in the test. The column was composed of the sediment layer and the aqueous layer. The sediment layer contained the upper half portion of the sediment collected from Taihu and its effective height was 0.15 m. Water collected from Taihu with an effective layer height of 1.30 m was carefully added, without disturbing the sediment. Algae count was operated by the Microscope counting method (Olympus BX41) (Huang et al., 1999). Then, different amounts of algae were added to creating algae-laden water with densities of 1.0×10^6, 1.0×10^7 and 1.0×10^8 cells/L (referred to as treatments 'L', 'M', and 'H', respectively). When the algae concentration was 1.0×10^8 cells/L, the thickness of the algal mats layer reached about 1.5 cm. According to the field investigation, OBWA occurred in the relatively stagnant water with a high temperature. Therefore, in this test, the algae decompositions were completed in the static conditions, with a constant temperature (environmental temperature of 30 ± 2 °C) in the dark.

2.2. Sample preparation and analytical methods

Samples were taken from the upper and lower layers of the water body, and the mean value was adopted. The upper layer was 15 cm under the surface of the water body and the lower layer was 15 cm above the bottom. In the initial period, the sampling interval was 1 day and was subsequently extended to 2 days in the latter period. The test lasted for 26 days.

The items investigated included DO, oxidation–reduction potential (ORP), Fe^{2+}, Mn^{2+}, sulfides, trace organics. DO and ORP were measured by a portable dissolved oxygen analyzer (DKK-TOA, DO-31P) and a pH/ION analyzer (DKK-TOA, IM-32P). Soluble Fe^{2+} and Mn^{2+} were measured with a flame atomic absorption spectrometer (Perkin Elmer, AAnalyst 400) after the water filtration with a 0.45 μm membrane (MEP, 2002). The sulfides were measured by amino dimethylaniline spectrophotometry (MEP, 2002).

Based on the findings of other studies (Chen et al., 2010; Yang et al., 2008) and the analysis of odorous compounds by GC–MS in the preliminary experiment, this study selected the following four organics to monitor, 2-methylisoborneol (2-MIB), β-cyclocitral, β-ionone, and dimethyl trisulfide (DMTS). Standards of the four organics were purchased from Supelco.

Organics analyses were performed by a purging and trapping (P&T) pretreatment and the gas chromatographic mass spectrometry (GC–MS, Thermo Scientific ITQ 1100TM). The procedures for the P&T pretreatment were detailed as follows: purging for 11 min at room temperature with a flow rate of 40 mL/min, blow drying for 1 min at 40 °C with a flow rate of 200 mL/min, thermal desorption for 2 min at 250 °C with a flow rate of 300 mL/min, and trap baking for 2 min at 270 °C after desorption. High-purity nitrogen (99.999%) was used as the purging gas. Organic compounds were separated with a Thermofisher TR-5 ms capillary column (30 m length × 0.25 mm i.d., 0.25 μm film thickness). The GC/MS conditions for the sample analysis were detailed as follows: the inlet port temperature was maintained at 250 °C and an unsplit stream sampling with a flow rate of 1.2 mL/min was applied. The temperature was first programed at 40 °C (hold for 3 min), raised to 250 °C (hold for 3 min) at a rate of 8 °C/min, and then raised to 280 °C (hold for 3 min) at a rate of 30 °C/min. The transfer line temperature and the ion source temperature were all set at 250 °C. The four organics were examined by the selected ion (SIM) method. The m/z valve and the retention time for each organic compound are shown in Table 1. All the analysis results were submitted to the rigorous control procedures. Blank samples, standard samples, and the duplicate samples were analyzed simultaneously in the experiment to provide quality control. The analytical precision, measured as relative standard deviation, was less than 8.0%.

3. Results and discussion

3.1. Sensory variation in simulated water

Treatments L and M showed no significant change in water color at any time (Table 2). Treatment H turned to a distinct black color from the 10th day, and the color darkened from the 12th to the 24th day before it spontaneously faded away. According to the olfactory analysis, treatment L did not emit the obvious odor. In contrast, treatments M and H emitted a strong smell starting from the 6th and the 4th day, respectively. In treatment H, there was a time difference between water stinking and blackening; the water began to emit a smelly odor and after approximately 6 days the water suddenly turned black.

Table 1 Retention time and m/z valve for every organic compound.

Organic compound name	Retention time (min)	m/z value selected
DMTS	8.72	126,45,79
β-cyclocitral	13.02	41,137
β-ionone	13.72	177,43
2-MIB	18.14	95,43,107

Table 2 Sensory variations observed in three treatments.

Treatment	L	M	H
Time for water to emit smelly odor (day)	None	6	4
Time for water to turn black (day)	None	None	10

3.2. Variation of DO and ORP

DO concentrations were obviously affected by algae and reduced sharply in the treatments L, M and H, during the incubation process (Fig. 2). On the 2nd day of the test, DO concentration in the treatment H reduced to 0 mg/L. On the 4th day, DO concentration in the treatment M reduced to 0 mg/L, with relatively slower oxygen consumption rate compared with that in the treatment H. DO concentration in the treatment L with the lowest algae density, reduced to 0 mg/L on the 10th day before the recovery thereafter.

The ORP values were affected by the algae densities and varied widely among the different treatments (Fig. 2). In the treatment H, the ORP value decreased rapidly from the beginning of the test to the 4th day (from 100 to −300 mV) and then stabilized at about −300 mV in the following days, which indicated a strong reducibility (Wang et al., 2004). It was concluded that the treatment H experienced a dramatic change from the aerobic condition to the anoxic and the anaerobic conditions in sequence. In the treatment M, the ORP value decreased to −100 mV on the 8th day and remained steady at about −100 mV from the 8th to the 14th day, which suggested a weaker reducibility than that of the treatment H. The ORP value in the treatment M then gradually increased to 0 mV after the 14th day. In the treatment L, the ORP value decreased gradually from the beginning of the test to the 10th day (from 130 to 0 mV) and then rose gradually. This implied that the treatment L maintained self-purification to some extent and reached equilibrium; furthermore, this sample showed no strong reducibility.

Low DO concentration was a typical characteristic of OBWA (Yang et al., 2008), but not all hypoxic areas with fairly low DO concentrations or hardly 0 mg/L would induce the OBWA (Table 2, Fig. 2). The same result was found in other waters. For instance, the hypoxia has been frequently reported in Kasumigaura (Fan et al., 1998), but OBWA has never been reported.

DO deficiency in the overlying water initiated a redox state change (Middelburg and Levin, 2009). The ORP levels would exactly represent the reduction potential of the water. In this study, when the ORP level reduced to −100 mV, the water emitted a smelly odor, and when the ORP level reduced to about −300 mV, the water broke out in OBWA. The phenomena may be attributed to the differences among the main microorganisms which contributed to the odor and black color.

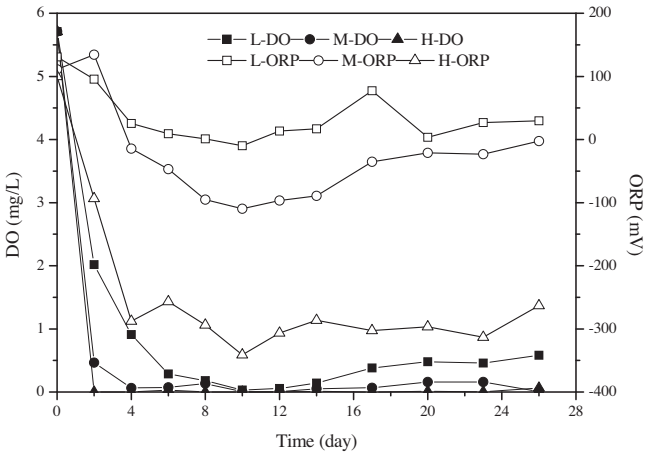

Figure 2 Change in dissolved oxygen (DO) and oxidation–reduction potential (ORP).

3.3. Production and variation of odorous organics

One of the main features of the OBWA is that the water body becomes strongly odorous. Not only the live algae produce volatile and non-volatile substances from the metabolic processes and cause odor (Lanciotti et al., 2003), but also the dead algae can cause odor (Nicholls et al., 1982). The decomposition of algal cells without cellulose cell wall allowed the release of intracellular matter into water including the odorous compounds. Meanwhile, algae might be utilized as a carbon source for actinomycetes which would produce odorous compounds (Sugiura et al., 1994). The decomposition of cyanobacteria blooms could produce large amounts of volatile sulfur compounds, including hydrogen sulfide, methyl mercaptan, dimethyl sulfide etc. (Zinder et al., 1977), isobutyl mercaptan (Jenkins et al., 1967), dimethyl trisulfide and β-cyclocitral (Jones and Korth, 1995; Juttner, 1984). As the major inorganic sulfide, hydrogen sulfide is one of the substrates to induce the occurrence of the black and odorous water (Zinder et al., 1977). Its formation was tracked with the sulfide concentration measurement as summarized in Fig. 5. The development dynamics of the four volatile organic compounds in different treatments are shown in Table 3.

2-MIB is a common odorous organic compound with a musty odor. Odor threshold concentration (OTC) for 2-MIB has been reported to range from 9 to 42 ng/L (Watson et al., 2000). 2-MIB was detected in the three treatments from the 8th to the 14th day of the test. The concentrations in treatments L, M and H were 6.3–8.9, 7.4–40.3 and 24.8–59.3 ng/L, respectively. The maximum value in treatment L was below the OTC. The maximum values in the treatments M and H were 4.5–6.6 times the odor threshold, which contributed to the generation of the water odor.

β-cyclocitral is an odorous organic compound which can generate different types of odor with a variation in its concentrations. The study indicated that its three odor descriptors, fresh grass, hay/woody, and tobacco-like smell, were observed at different concentration ranges: 0.5– μg/L1, 2–20 μg/L and above 20 μg/L, respectively (Young et al., 1999). From the 2nd to the 6th day of the test, β-cyclocitral was detected in the three treatments, with its concentration far less than 0.5 μg/L. Therefore, it was speculated that β-cyclocitral contributed little to the production of the odorous water.

High β-ionone concentrations were detected from the 4th to the 12th day in the three treatments, reaching a maximum value of 1.7, 19.7 and 96.7 times its OTC value (7 ng/L) (Cotsaris et al., 1995), respectively. This indicates that β-ionone was more important for the production and release of intense odor than β-cyclocitral. However, it was worth the attention that β-cyclocitral at a low concentration was detected at the beginning of the test and before β-ionone. There was no significant correlation between β-cyclocitral and β-ionone concentrations. It was different from the spot survey results from the Gonghu Bay of Taihu conducted by Jun Chen, etc. (Chen et al., 2010; Ma et al., 2013). Because the probable reason was that β-cyclocitral was derived from β-carotene and the cleavage reaction was oxygen dependent (Young et al., 1999).

DMTS is a volatile sulfur compound that smells like rot swamp. In the treatment L, a very low concentration of DMTS (below its OTC level) was detected only from the 9th to the 11th day of the test. In the treatment M, DMTS was detected from the 6th to the 10th day, and its maximum value was 35 times of its OTC level on the 8th day. In the treatment H, DMTS was detected from the 4th to the 12th day, and its maximum value was 449 times of its OTC level on the 8th day.

Through the comparison of the ratios of the test organics concentrations to their OTC in the three treatments, it was concluded that the water with a density of 1.0×10^7 cells/L would emit an appreciable odor. 2-MIB and β-cyclocitral concentrations were below or slightly higher than their OTC levels and were less important for the odor emissions of the three treatments. Compared to β-ionone, DMTS had more intensive effect on the odor emissions regardless of the absolute concentrations or the ratio of the concentration to its OTC. Therefore, it was concluded that DMTS was the primary contributor to the odor emission in the treatments M and H. β-ionone was the second important source of the smell in the water samples.

According to the sample analysis, microcystis accounted for 95% of the algae in the test material, originated from Taihu. It was found that DMTS could be generated by microcystis, especially during the process of microcystis decomposition (Juttner and Hoflacher, 1985). In this study, the decomposition of cyanobacteria blooms consumed a large amount of DO and rapidly created the anaerobic conditions in the water environment. Under anaerobic conditions, many bacteria can convert sulfurated methionine and cysteine in the organisms into sulfureous organics with a small molecular size, such as DMTS and other thioether matters (Yu et al., 2007). Thus, the algae with high density and the anaerobic environment formed during the algae accumulation and decomposition provided the necessary conditions for the generation of odorous matters.

3.4. Variation of Fe^{2+}, Mn^{2+} and sulfide

Metal sulfides were considered the main blackening matter of the 'Black Water Cluster' (Liu et al., 2009). However, there was a lack of systematic data to verify this statement. Meanwhile, the conditions required for OBWA formation are not clear, which caused the development limitation of OBWA prevention and control technologies.

Table 3 Variation of 2-MIB, β-cyclocitral, β-ionone and DMTS concentration in three treatments Unit: ng/L.

	2-MIB			β-cyclocitral		
	L	M	H	L	M	H
0d	—	—	—[a]	—	—	—
1d	—	—	—	—	—	—
2d	—	—	—	—	16.7 ± 4.7	34.2 ± 4.9
3d	—	—	—	—	22.3 ± 5.1	29.1 ± 3.2
4d	—	—	—	11.2 ± 3.5	23.1 ± 4.9	21.6 ± 2.7
5d	—	—	—	—	—	18.3 ± 5.5
6d	—	—	—	—	—	11.2 ± 4.1
7d	—	—	—	—	—	—
8d	—	7.4 ± 1.6	—	—	—	—
9d	—	10.8 ± 1.3	36.7 ± 3.4	—	—	—
10d	6.3 ± 0.9	40.3 ± 2.5	59.3 ± 2.9	—	—	—
11d	7.4 ± 1.8	31.5 ± 1.9	43.1 ± 2.7	—	—	—
12d	8.9 ± 0.7	20.5 ± 0.6	24.8 ± 5.2	—	—	—
13d	6.8 ± 1.1	19.7 ± 2.3	29.8 ± 2.6	—	—	—
14d	7.1 ± 2.4	16.5 ± 1.2	35.6 ± 3.7	—	—	—
15d	—	—	—	—	—	—
16d	—	—	—	—	—	—
	β-ionone			DMTS		
	L	M	H	L	M	H
0d	—	—	—	—	—	—
1d	—	—	—	—	—	—
2d	—	—	—	—	—	—
3d	—	—	—	—	—	—
4d	—	—	11.2 ± 1.5	—	—	218.9 ± 35.6
5d	—	—	9.5 ± 0.6	—	—	1089.5 ± 166.5
6d	7.2 ± 0.7	13.2 ± 1.4	10.6 ± 0.8	—	187.3 ± 14.9	2071.1 ± 98.7
7d	7.5 ± 1.3	17.2 ± 1.3	42.8 ± 8.3	—	265.4 ± 22.5	3269.4 ± 159.2
8d	10.8 ± 2.4	22.2 ± 2.6	87.6 ± 9.2	—	350.9 ± 29.7	4489.5 ± 349.7
9d	11.7 ± 0.5	69.4 ± 5.9	676.8 ± 15.6	6.4 ± 1.9	119.4 ± 10.4	2281.6 ± 191.7
10d	12.0 ± 1.1	137.7 ± 10.4	339.1 ± 29.7	8.9 ± 2.4	90.8 ± 7.2	452.1 ± 58.7
11d	8.9 ± 0.8	84.2 ± 7.2	181.6 ± 13.4	5.9 ± 0.6	—	268.3 ± 48.2
12d	5.6 ± 0.4	66.1 ± 5.8	85.7 ± 9.2	—	—	82.1 ± 10.3
13d	—	—	—	—	—	—
14d	—	—	—	—	—	—
15d	—	—	—	—	—	—
16d	—	—	—	—	—	—

[a] Not detected.

Fe in the simulated water samples was mainly from the collected Taihu water, and existed in dissolved and particulate forms with the latter as the dominant form (Zhu et al., 2005), in which the latter was dominant. As shown in Fig. 3, the concentration of dissolved Fe^{2+} (referred to as 'D-Fe^{2+}', below) showed a small fluctuation in the treatments L (0.006–0.117 mg/L, mean 0.048 mg/L) and M (0.020–0.132 mg/L, mean 0.072 mg/L). Additionally, there was no obvious increase or decrease throughout the test. The D-Fe^{2+} concentration of the treatment H continued to increase at the initial stage of the test, reached a peak concentration of 0.326 mg/L on the 8th day, and then decreased. Fe mainly exists in the form of Fe–Mn oxide compounds in Taihu (Chi et al., 2007), and the compounded metallic bond deoxidizes to release metal when DO is badly depleted or when ORP decreases. In this study, the ORP value decreased to −100 mV on the 2nd day and then to −300 mV on the 4th day. The strong reducing conditions contributed to the possibility of Fe reducibility (Beutel et al., 2008; Davison, 1993),

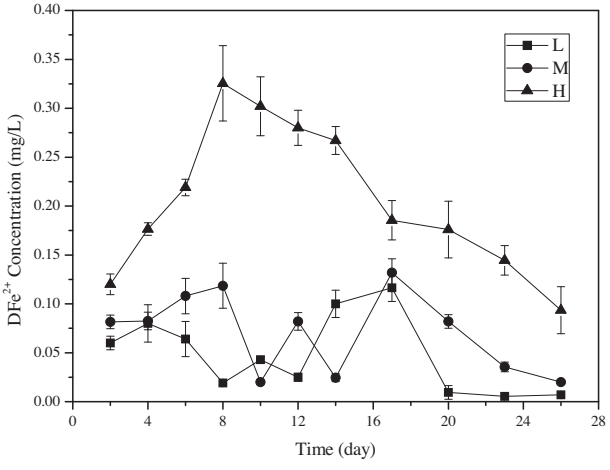

Figure 3 Variations of D-Fe^{2+} in different treatments.

existing in the form of Fe–Mn oxide compounds. Hence, D-Fe^{2+} concentration showed an increasing trend at the initial stage of the test. Later, it decreased probably because Fe^{2+} and S^{2-} in the water samples combined to produce FeS and other undissolvable matters (Balistrieri et al., 1992; Hamilton-Taylor et al., 2005). Compared with the variation of D-Fe^{2+} concentration after the 14-day incubation, D-Fe^{2+} concentration from the 8th to the 14th day decreased slowly. Therefore, it was speculated that from the 8th to the 14th day, an amount of D-Fe^{2+} was released for combination.

Mn in the simulated water samples was mainly from the collected Taihu water. Mn in the suspended solids existed in the water-soluble and exchangeable form and the Fe–Mn oxide form, which took equal proportion (Chi et al., 2007). Mn in the water-soluble and exchangeable form can be released under a neutral condition. As shown in Fig. 4, the concentration of the dissolved Mn^{2+} (referred to as 'D-Mn^{2+}' below) in the treatments L and M showed a slight increasing trend at the initial stage. In treatment H, because of a reductive atmosphere, not only Mn existing in the water-soluble and exchangeable form but also in the Fe–Mn oxide form was released. Thus, the concentration of D-Mn^{2+} showed a significantly increasing trend. On the 14th day, it reached 0.196 mg/L and then decreased. Iron sulfide and Manganese sulfide were considered as the blackening causing substances, but D-Mn^{2+} began to decrease later than D-Fe^{2+} during the test period. The formation of FeS is expected to precede MnS, because its solubility product constant is lower than MnS (Fang et al., 2005).

In water, sulfide is composed of dissolvable H_2S, HS^- and S^{2-}, soluble sulfides in suspended solids, acid soluble metal sulfides and unionized organic or inorganic sulfides. The sulfides involved in this test included dissolvable inorganic sulfides and acid soluble metal sulfides. Sulfides in the treatments L and M maintained a low level at the initial stage of the test, gradually increased from the 8th day, reached a peak (0.12 mg/L, 0.19 mg/L, respectively) on the 10th day and then slowly decreased during the latter stage of the test. Sulfide in the treatment H began to increase from the 8th to the 23rd day (maximum, 1.45 mg/L) and slightly decreased thereafter.

Protein is the main sulfur-containing organic in algae. During the decomposition of dead algae, sulfur-containing proteins are released into the water and are split into macromolecular sulfur-containing organics with the help of hydrolytic bacteria. Research has indicated that sulfur-containing amino acids, such as methionine, cysteine and cystine could be decomposed into sulfide by microorganisms (Banwart and Bremner, 1975; Yang et al., 2008). Methionine was considered an important precursor in the production of volatile organic sulfur compounds such as DMTS, which was broken down into hydrogen sulfide in the lakes suffering from cyanobacteria blooms (Lu et al., 2013). Thus, a higher concentration of organic sulfides (DMTS) was detected at the initial stage of the test (Table 3); thereafter, the concentration of inorganic sulfide increased (Fig. 5).

Inorganic sulfide is generated from the decomposition and conversion of sulfur-containing organics, and from the reduction of sulfate and other matters by sulfate reducing bacteria (SRB) under anaerobic conditions (Holmer and Storkholm, 2001). There is a great diversity of bacteria in the lake sediment (Ye, 2009) where SRB is often detected survival under the anaerobic conditions. In the treatment H, up to the 8th day, the ORP value dropped to −360 to 300 mV (Fig. 2), the aquatic environment which is conducive to SRB metabolism and sulfate reduction (Xu et al., 2009). Thus, it would promote the sulfur reduction and accelerate sulfide release (Roden and Tuttle, 1992).

3.5. *Analysis of OBWA formation*

The two most important and direct characters of OBWA are black color and smelly odor. However, not all water emitting odor would turn black. Thus, water blackening is considered a direct evidence of OBWA formation.

The black color of the test water was continuous with an increase of sulfide concentration. In the treatment H, DO was consumed rapidly and its concentration sharply dropped to 0 mg/L, along with the massive accumulation and death of cyanobacteria. Meanwhile, the water body was rapidly converted from an aerobic state into a hypoxic state (Bianchi et al., 2010), continuing to deteriorate until reaching an anaerobic state. On the 8th day of the test, the ORP value fell sharply to −300 mV. Under an aerobic reductive condition, Fe with a high valence from the particulate matter and from the dead algae of Taihu was restored to that of a low valence and then released into the overlying water (Couture et al., 2010). From the 8th day, the D-Fe^{2+} concentration of the

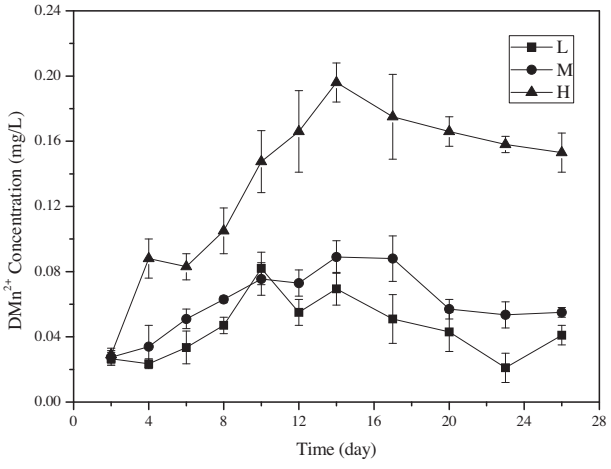

Figure 4 Variations of D-Mn^{2+} in different treatments.

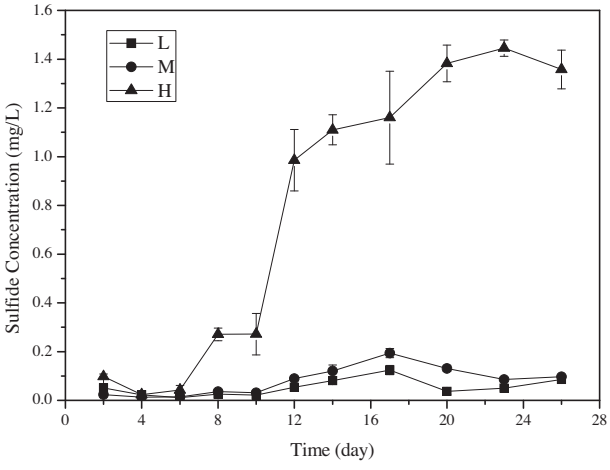

Figure 5 Variations of sulfide in different treatments.

treatment H began to decrease, meanwhile, the sulfide concentration increased significantly and reached 0.302 mg/L on the 10th day, when the water became obviously black. It can be presumed that the formation of algae-induced OBWA is closely related to the increase of sulfide, which would combine with Fe^{2+}, Mn^{2+} and form the black substance found in the water body. The study also indicated that the reductive environment facilitated the formation of black metal sulfides represented by FeS (Liu et al., 2009; Stahl, 1979).

Sulfur transformation played an important role in the formation of OBWA and is responsible for the formation of both the odorous matter and the main substance causing the blackening. According to the comprehensive analysis of the odorous matters in the treatments L and M, and the blackening substance in the treatment H, it is concluded that in a reductive environment with an ORP value of −250 to 50 mV, sulfur-containing organics (represented by DMTS) formed. In a strong reduction environment with an ORP value of −350 to 300 mV, the sulfur ions were generated from further degradation of sulfur-containing organics and from the reduction of sulfate combined with Fe^{2+} and other metal irons that were released from the Fe–Mn oxides. The metal sulfides formed the black substance found in the water bodies. The oxidation-deoxidization conditions for the odorous and black matter formation varied; the water first became odorous and then blackened. When the water ORP value decreases to about −300 mV or the algae density reaches 1.0×10^8 cells/L, OBWA can be expected to occur.

4. Conclusions

High density of algae is a major factor to trigger OBWA formation. The massive accumulation and the death of algae provided required substances for OBWA. There was a time difference between water stinking and blackening during the formation of OBWA with the stinking first. Although stinking was not always accompanied by blackening, blackening was often accompanied by stinking.

Different ORP values revealed the corresponding conditions for the formation of stinking and blackening in a water body. In an anaerobic environment, when the ORP value was between −250 and −50 mV, along with the death and degradation of algae, DMTS and other odorous organics were produced. When the ORP value was between −350 and −300 mV, waters showed an extremely strong reducing environment, which led to the reduction and release of heavy metal ions, for water blackening because of the metal sulfide formation. Therefore, the water ORP value below −300 mV can be considered as the indicator for OBWA formation.

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