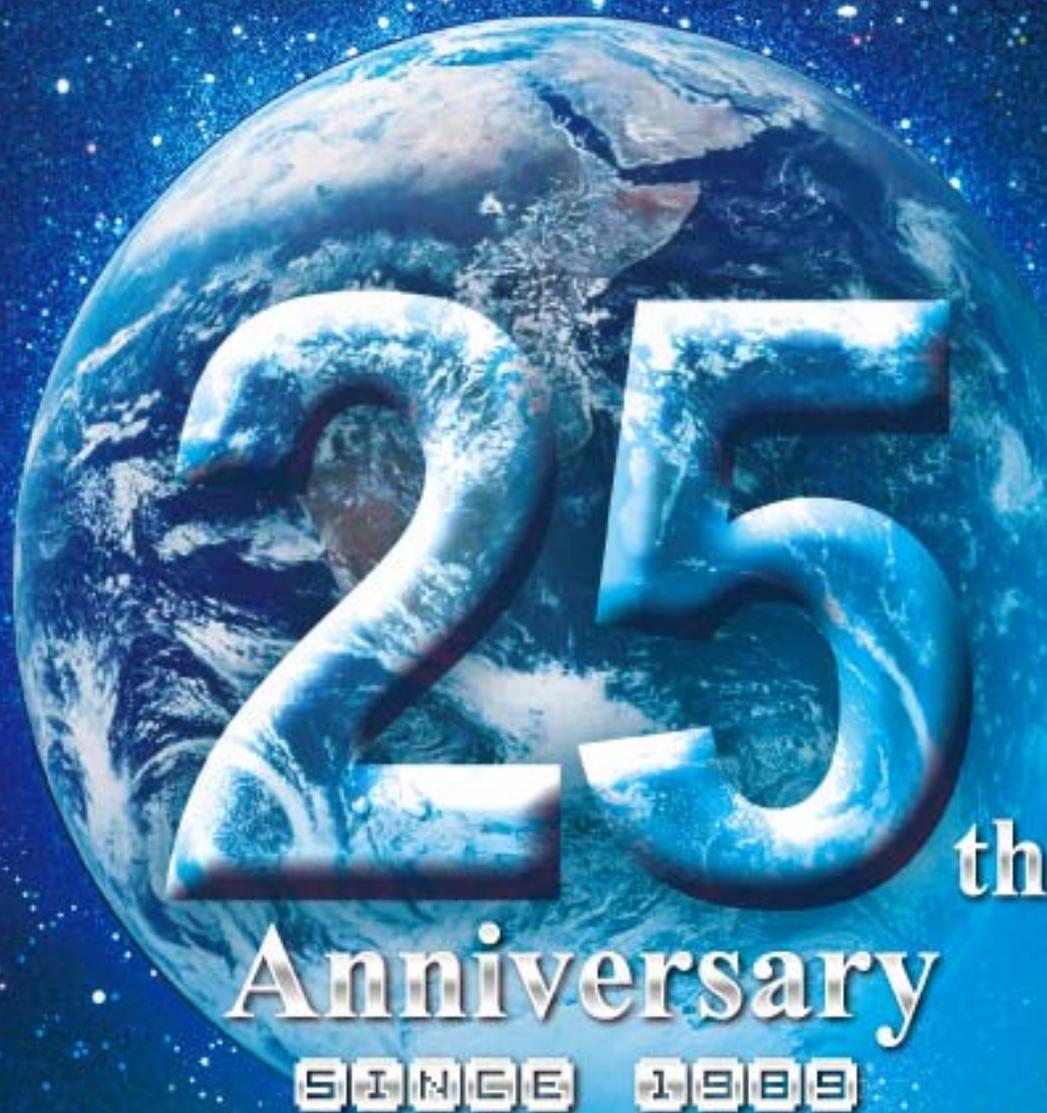


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## Sulfur-containing amino acid methionine as the precursor of volatile organic sulfur compounds in alga-induced black bloom

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### Abstract

After the application of methionine, a progressive and significant increase occurred in five volatile organic sulfur compounds (VOSCs): methanethiol (MeSH), dimethyl sulfide (DMS), dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS) and dimethyl tetrasulfide (DMTeS). Even in the untreated control without a methionine addition, methionine and its catabolites (VOSCs, mainly DMDS) were found in considerable amounts that were high enough to account for the water's offensive odor. However, blackening only occurred in two methionine-amended treatments. The VOSCs production was observed to precede black color development, and the reaching of a peak value for total VOSCs was often followed by water blackening. The presence of glucose stimulated the degradation of methionine while postponing the occurrence of the black color and inhibiting the production of VOSCs. In addition, DMDS was found to be the most abundant species produced after the addition of methionine alone, and DMTeS appeared to be the most important compound produced after the addition of methionine+glucose. These results suggest that methionine acted as an important precursor of the VOSCs in lakes suffering from alga-induced black bloom. The existence of glucose may change the transformation pathway of methionine into VOSCs to form larger molecular weight compounds, such as DMTS and DMTeS.

**Key words:** algal blooms; black bloom; methionine; volatile organic sulfur compounds; sulfur-containing amino acid

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### Introduction

Volatile organic sulfur compounds (VOSCs) have caused great concern due to their offensive odor, low sensory threshold and important role in atmospheric chemistry (Kiene and Hines, 1995; Andreae and Crutzen, 1997; Kiene and Linn, 2000). VOSCs have been found in marine, freshwater and terrestrial systems (Muezzinoglu, 2003; Yi et al., 2008). The production of VOSCs in a natural aquatic environment, especially in marine and oxic freshwater systems, is now widely studied (Ginzburg et al., 1999; Gun et al., 2000; Hu et al., 2007; Reese and Anderson, 2009). The mechanisms underlying VOSCs formation are quite different. One such mechanism is the degradation of certain organosulfur compounds, most notably the alga-derived compound dimethylsulfoniopropionate (DMSP), which leads to the formation of DMS in surface ocean waters and *Spartina alterniflora* marshes (Dacey et al., 1987; Kiene and Linn, 2000; Xie et al., 1998). DMSP acts as an osmoprotective agent (osmolyte) in many algae,

bacteria and some plants living in marine environments. It can be present in relatively high concentrations (e.g., 100–400 mmol/L) in marine macro- and microalgae (Keller et al., 1989; Kiene and Taylor, 2002). Second, the methylation of thiols in anaerobic sediments is also an important mechanism of VOSCs formation that occurs in a variety of oxic freshwater habitats (Finster et al., 1990; Stets et al., 2004). Polysulfides are direct precursors for VOSCs; the mechanism of VOSC formation likely involves the biogenic production of inorganic polysulfides, which then produce dimethylpolysulfides after methylation by organic methyl donors (Drotar et al., 1987; Ginzburg et al., 1999; Gun et al., 2000). Third, previous studies have indicated that VOSCs can arise from the microbiological degradation of sulfur-bearing amino acids under either anaerobic or aerobic conditions in sediments or terrestrial systems (Kiene and Visscher, 1987, 1988; Andreae and Jaeschke, 1992; Wu et al., 2010). However, the mechanisms that lead to VOSC production mentioned above might be quite different from those produced in the 2007 water crisis in Wuxi, China, which was an extreme paroxysmal water

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pollution event in which “black bloom” formed under different ambient conditions, organic burdens, microbial groups and enzyme activity levels.

In recent years, algal blooms in eutrophic freshwater lakes during warmer seasons have been a serious environmental and ecological problem, such as in Lake Kasumigaura (Sugiura and Kazunori, 2000), Lake Garda (Pucciarelli et al., 2008) and Lake Taihu (Yang et al., 2008; Zhang et al., 2010). However, eutrophication could have been significantly worse for Lake Taihu. The issue that drew public attention to Lake Taihu during the Wuxi water crisis during the summer of 2007 was the black bloom that was formed through algal blooms. Although the term black bloom was first suggested during this event, similar problems had been observed years earlier, but there was less concern due to lesser effects on water supplies than during the 2007 crisis (Yang et al., 2008). A black color and foul odor are two essential characteristics of the black water agglomerate. The chemicals responsible for the foul smell in the tap water were later identified as VOSCs. Yang et al. (2008) found that dimethyl trisulfide (DMTS), which occurred in concentrations up to 11.4  $\mu\text{g/L}$ , was an abundant VOSC species responsible for the foul smell in tap water. By comparison, Zhang et al. (2010) indicated that methanethiol (MeSH, 204  $\mu\text{g/L}$ ) and demethyl (DMS, 93.9  $\mu\text{g/L}$ ) were the dominant VOSCs in an earlier stage of black bloom and then later turned into the more oxidized dimethyldisulfide (DMDS, 46.1  $\mu\text{g/L}$ ) and DMTS (17.2  $\mu\text{g/L}$ ). These contaminants originated from the decomposition of a massive cyanobacterial bloom that was triggered by illegal industrial discharges and inadequately regulated domestic pollution. The decomposition of dead algae combined with the high pollution load resulted in extremely anaerobic conditions. Fe-Mn oxides and sulfides at the water-sediment interface were then deoxidized and released rapidly to combine with hydrogen sulfide that was produced abundantly in the strongly reducing environment. The resulting black metal sulfides were distributed in the overlying water and blackened the water. In summary, the duration of this black bloom was connected to high ammonium levels, low redox potential (Eh), odorous VOSCs and a dark appearance, and the agglomerate affected the aesthetics and drinking quality of the water of surrounding cities. There have been many studies concerning the production of VOSCs from various sources, including food aroma compounds produced during fermentation (Blank, 2002; Landaud et al., 2008), agricultural operations and food industries producing waste gases (Rappert and Müller, 2005; Kim et al., 2009) and various waste treatment processes, such as landfilling (Kim et al., 2005, 2006) and composting (Vandergheynst et al., 1998; Smet et al., 1999). However, research on the production of VOSCs in the alga-induced black bloom is far from sufficient. The agglomerate's random occurrence is not well understood, and better analyses

and detection methods are needed. It is indicated that many bacteria (e.g., *Pseudomonas* sp.) may produce VOSCs by breaking down the amino acids methionine and cysteine into hydrogen sulfide, methanethiol and dimethylpolysulfides during the process of black bloom formation (Yang et al., 2008). Sulfur-containing amino acids are commonly found in plants, animals, microorganisms and blue-green algae. The component analysis of blue-green algae in Lake Taihu showed a high sulfur-containing amino acid content of up to 0.97% of dry weight (Li, 2009). Because L-cysteine has a lower concentration than L-methionine in blue-green algae, and the main product of catabolism is hydrogen sulfide, cysteine was not considered in the production of VOSCs. Therefore, the methionine derived from algal decomposition was speculated to be a potential precursor of the VOSCs in the alga-induced black bloom.

Until now, no empirical research has been carried out to verify methionine as a precursor of VOSCs and the concomitant occurrence of black bloom in an aquatic environment suffering from algal blooms, and the responsible mechanism is still debated due to a lack of sufficient and convincing evidence. Because people lack information on the nature of black bloom, controlling measures, including aeration and casting oxidants into the water, have not been able to effectively prevent it, especially given that these measures tend to be adopted primarily after the occurrence of black water agglomerate. This article aims to clarify the precursors of VOSCs in algal bloom-induced black bloom as well as to investigate the production mechanism of VOSCs and the concomitant process of black bloom development in the Lake Taihu aquatic environment under strictly controlled conditions.

## 1 Materials and methods

### 1.1 Site description and sample collection

The sediment and water used for this study were collected from Yueliang Bay, Lake Taihu, China in September of 2010 after algal blooms disappeared. Sediment was collected using a core sampler with a diameter of 8 cm and a length of 25 cm, along with water from the overlying water column. Lake water was collected in polyethylene bottles. The sampling site (31°24'35.8"N, 120°6'4.6"E) is located in the northern near-shore area of Lake Taihu, where algal blooms and black water agglomerate have often appeared during the late spring and summer of recent years (Fig. 1). The upper 6 cm of sediment was used in the experiments. This part of the sediment had the following physical and chemical parameters: total nitrogen (TN) 1268.48 mg/kg; total phosphorus (TP) 635.43 mg/kg; dissolved oxygen (DO) 5.6 mg/L; pH 7.3; Mn 387 mg/kg, Fe (Oxal-Fe) 3400 mg/kg and acid volatile sulfide (AVS), 125 mg/kg. The concentration of  $\text{SO}_4^{2-}$  in the interstitial water of the sediment was approximately 50 mg/L. In

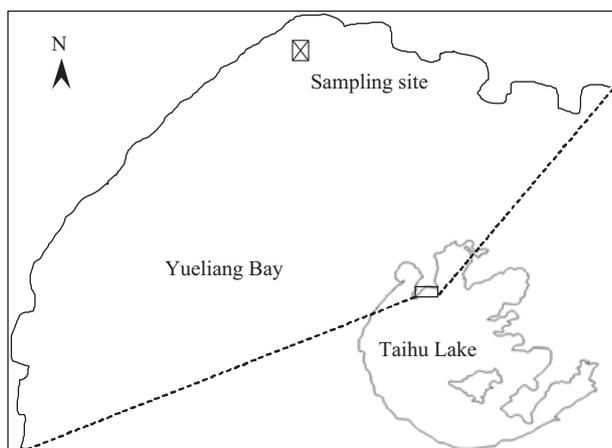


Fig. 1 Location of the sampling site in Yueliang Bay, Lake Taihu, China.

the laboratory, overlying water was siphoned from each core. The uppermost 6 cm of sediment was sliced and homogenized with foreign matter extracted, then placed immediately into jars, filled to the top, sealed with airtight lids and stored in a refrigerator (4°C) before use. The lake water was filtered through a microfiber membrane filter (0.45 μm pore size).

### 1.2 Experimental procedures

The laboratory simulations employed self-made glass reactors with a capacity of 6 L (Fig. 2). The reactors consisted of two connected glass spheres containing a liquid sampling outlet close to the sediment-water interface in the lower sphere, a gaseous sampling outlet at the top of the upper sphere and a measurement hole located in the upper sphere below the gaseous sampling outlet to minimize gas exchange with the atmosphere or loss to headspace during measurements. The experiment was conducted in duplicate. Upon beginning the experiment, each reactor was loaded with 600 g of homogeneous slurries. Additions were prepared in lake water. These additions included methionine (final concentration 1.0 g/L) and a mixture of methionine and glucose (both in 1.0 g/L final concentration). Untreated controls were obtained by the addition of lake water alone. For each reactor, approximately 7 L of solution was used to ensure that the water surface was slightly below the gaseous sampling outlet to reduce the headspace but as high as possible above

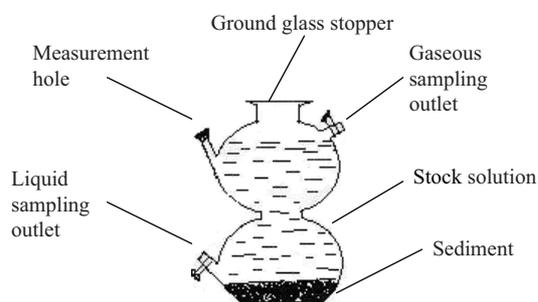


Fig. 2 Schematic diagram of the self-made airtight reaction system.

the measurement hole to reduce gas emissions during measurement. The apparatus was then tightly capped and incubated undisturbed at  $25 \pm 3^\circ\text{C}$ , which is the mean *in situ* temperature measured for black bloom events in the field. The whole experiment lasted for 22 days, and samples were collected at intervals of 1–3 days. Water samples were collected in 40 mL glass bottles that were carefully filled, closed without headspace and stored in a refrigerator at 4°C until analysis. Gas samples were collected in a Teflon gas sampling bag. Analyses were completed within 24 hr of sampling.

### 1.3 Analytical methods

In this study, the extraction and analysis of VOSCs in water samples were conducted using an automatic SPME method and an Agilent 7890A gas chromatography-mass selective detector (GC-MSD, Agilent Technologies, USA). Defrosted samples (20 mL) kept at 4°C were pre-incubated for 10 min at 30°C. The extraction was conducted with a 50/30 μm DVB/carboxen-PDMS fiber (Supelco, No. 57348-U, USA) for 30 min at 65°C with agitation at 150 r/min. After desorption at 250°C for 60 sec in the chromatograph injection port, volatiles were transferred onto a capillary column (HP-5 MS, 30 m × 0.25 mm × 0.25 μm, Agilent Technologies, USA) swept by helium at a constant flow rate (1.0 mL/min). The GC oven temperature was initially programmed at 45°C and held for 3 min; then, it was increased to 200°C at 10°C/min, where it was held for 3 min. The MSD was run in scan mode with a mass range of 35–250 amu. The electron impacting ionization method was used. Volatile compounds were identified according to the chromatograms of their ions and were quantified from calibration curves established with pure standard chemicals. The concentrations of MeSH, DMS, DMDS and DMTS were quantified using the corresponding standard calibration curves; DMTS standards were used as an alternative standard for DMTeS given that no commercial products were available. The detection limit was in the range of 2.2–4.0 ng/L for the five VOSCs.

Gas samples were measured by an Agilent 7890A gas chromatography system equipped with a flame photometric detection (FPD) system operated in the sulfur mode to detect gaseous VOSCs, as well as with a flame ionization detector (FID) equipped with a nickel reforming system to analyze CH<sub>4</sub> and CO<sub>2</sub>. The GC-FPD injection was made in the splitless mode at 120°C. The temperature of the detector was 250°C, and it was fed with 50 mL/min of hydrogen, 65 mL/min of synthetic air and 30 mL/min of helium as auxiliary gas. Separation was performed using a GAS-PRO capillary PLOT column (60 m × 0.32 mm). Helium was used as the carrier gas with a constant flow rate of 3.0 mL/min. The column oven temperature was regulated as follows: it was first held at 50°C for 5 min, then programmed to rise at 25°C/min to 250°C, where it was held for 7 min. The GC-FID injector temperature was set to

100°C. The oven temperature was 40°C, the temperature of the detector was 180°C, and the temperature of the nickel reformer was set to 350°C.

Methionine was analyzed using the sodium nitroprusside method (Timothy et al., 1941). Microbial activity was measured with the modified fluorescein diacetate method (FDA) that measures the amount of enzymatic activity related to organic matter decomposition (Stubberfield and Shaw, 1990). Ten milliliters of the liquid samples was placed into centrifuge tubes. After 1 mL of 2 g/L FDA was added, the tubes were shaken at 30°C for 3 hr. Then, the reaction was halted by the addition of a formaldehyde/trichloromethane solution (2/1, V/V). The suspension was centrifuged before the absorbance was measured at 490 nm with a UV-Visible Shimadzu UV-2100 (Japan) spectrophotometer. Microbial activity was expressed as  $\mu\text{g}$  FDA/(hr·mL). The platinum electrodes were connected to a millivoltmeter to measure Eh of overlying water *in situ* through the measurement hole.

#### 1.4 Chemicals

L-Methionine, MeSH, DMS, DMDS and DMTS were obtained commercially from Sigma-Aldrich and were of the highest purity available. Standard forms of the gases MeSH, DMS, DMDS, CH<sub>4</sub> and CO<sub>2</sub> were purchased from the Dalian Date Gas Co. Ltd., China. Other routine chemicals were purchased from the Sinopharm Chemical Reagent Co., China and were of analytical grade purity.

## 2 Results

### 2.1 Development of the black bloom

The water column and sediment remained unchanged in untreated controls at all times. The two methionine addition treatments turned black and produced air bubbles. However, the occurrence times of the black color and the evolution of gases differed greatly between the two treatments (Table 1). Gas evolution from the methionine+glucose treatment began on day 2 with a break of 4 days (from day 6 to day 9) and remained constant thereafter. By comparison, gas evolution from the methionine alone treatment began on day 14. As a whole, gas emissions from the methionine+glucose treatment greatly exceeded those of the methionine alone treatment. However, the sediment and overlying water of the methionine alone treatment turned black on day 14, which was earlier than the blackening of the methionine+glucose combination on day 20. Notably, for all treatments, after the blackening reached a climax, the black color spontaneously faded away with incubation time in the closed system.

### 2.2 Fluctuations in redox potentials over time

Redox potentials for the overlying water during the experiment were monitored and are shown in Fig. 3. In untreated

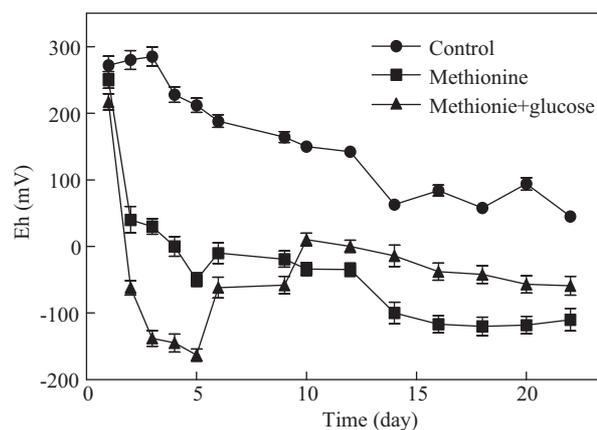
**Table 1** Sensory variations observed in the black bloom development processes

Treatment	Time for sediment and water to turn black (day)	Time until black water agglomerate occurrence (day)	Start of gas production (day)	Total volume of gas produced (mL)
Control	None	None	None	None
Methionine	9	14	14	300
Methionine +glucose	12	20	2	1000

controls, Eh decreased steadily from beginning to end. The redox potentials decreased rapidly at the second day in the methionine alone treatment (from 245 to 45 mV) and in the methionine+glucose treatment (from 217 to -62 mV). Then, Eh for the methionine alone treatment remained steady from day 2 to day 9, decreasing gradually. However, for the methionine+glucose treatment, Eh continually decreased from the beginning to day 5 (from 245 to -163 mV), then rose from day 5 to day 10 (from -163 to 0 mV), and gradually decreased thereafter.

### 2.3 Dynamics of microbial activity in overlying water adjacent to the water-sediment interface

Because sediment sampling could not be carried out in the airtight reaction system, microbial activity in the overlying water adjacent to the water-sediment interface was determined as a substitute. As presented in Fig. 4, microbial activity was very low for all treatments during the early stage of incubation. Then, the microbial activity of the untreated controls decreased slightly and was relatively low compared with those of the methionine alone and methionine+glucose treatments, which significantly increased over time despite some fluctuations. Overall, the methionine+glucose treatment showed higher microbial activity than the methionine alone treatment.



**Fig. 3** Change in redox potentials (Eh) during the metabolism process. The error bars represent the standard deviation of two replicates.

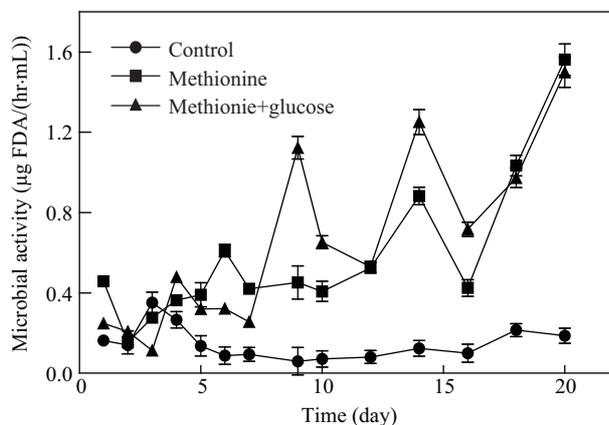


Fig. 4 Variation in microbial activity during the metabolism process.

## 2.4 Degradation of methionine

To assess the degradation rate of methionine, the methionine residual in the water column was measured. As shown in Fig. 5, even in the untreated control, methionine was present in small amounts at the beginning of the incubation and increased with time over the first 3 days. It then disappeared from the water after 6 days of incubation. For the methionine alone and methionine+glucose treatments, methionine remaining in the water column decreased gradually over the first 6 days. The turning point appeared between day 6 and 7, when the degradation rate rapidly increased at an identical rate for the methionine alone and methionine+glucose treatments. In addition, gas emissions from the methionine+glucose treatment were temporarily suspended and then began again 3 days later. Then, the methionine degradation rate rapidly increased over time. Overall, the degradation rate of methionine in the methionine+glucose treatment was greater than in the methionine alone treatment over the whole catabolism process.

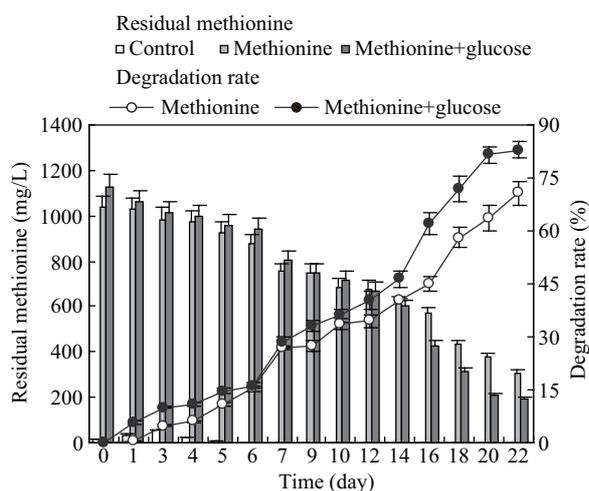


Fig. 5 Variation in residual methionine in different treatments and the corresponding degradation rate.

## 2.5 Volatile products from methionine during the catabolism process

Figure 6 displays a typical chromatogram of VOSCs produced during the incubation period. Five VOSCs including MeSH, DMS, DMDS, DMTS and DMTeS, were detected in the water samples of the present study. The untreated control also had a certain amount of VOSCs produced. Addition of methionine resulted in the rapid accumulation of total VOSCs with a peak on day 9 at 907.4 µg/L (Fig. 7f). Addition of methionine+glucose showed a slightly higher amount, 921.3 µg/L, on day 12. In general, the production of individual VOSC species increased in the early stage and then declined with incubation time after reaching a maximum. However, the production patterns of the five VOSC species were quite dissimilar for the different treatments in this study (Fig. 7).

MeSH was below detection limits for the untreated control and methionine alone treatment and reached 0.061 µg/L for the methionine+glucose treatment in the first 2 days. After that, MeSH was detected at low concentrations at all times for the untreated control, gradually increased for the methionine alone and methionine+glucose treatments before day 12 and then eventually decreased (Fig. 7a). DMS was present in all samples at the beginning of incubation. DMS associated with the addition of methionine alone was less than that of methionine+glucose but much greater than that of the untreated control (Fig. 7b). DMDS, DMTS and DMTeS were detected in all of the samples of the methionine alone and methionine+glucose treatments. DMDS evolved from the methionine alone treatment was consistently greater than that from the methionine+glucose treatment; it was far greater than that of the untreated control (Fig. 7c). DMTS associated with methionine+glucose and methionine alone treatments did not differ (Fig. 7d). For the untreated control, little DMTS and DMTeS were found in water samples with the exception of a few points. DMTeS formed in large amounts in the methionine+glucose treatment at a rate approximately two times greater than in the methionine alone treatment (Fig. 7e).

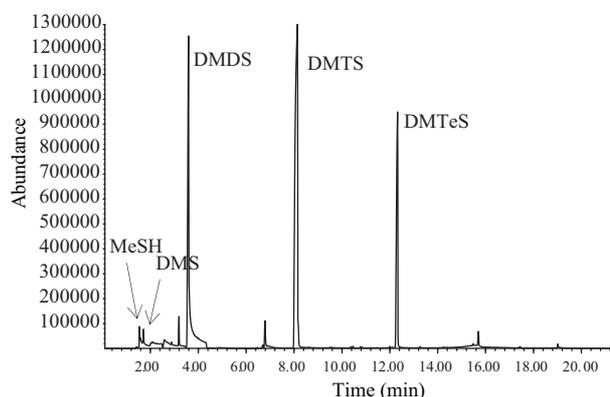
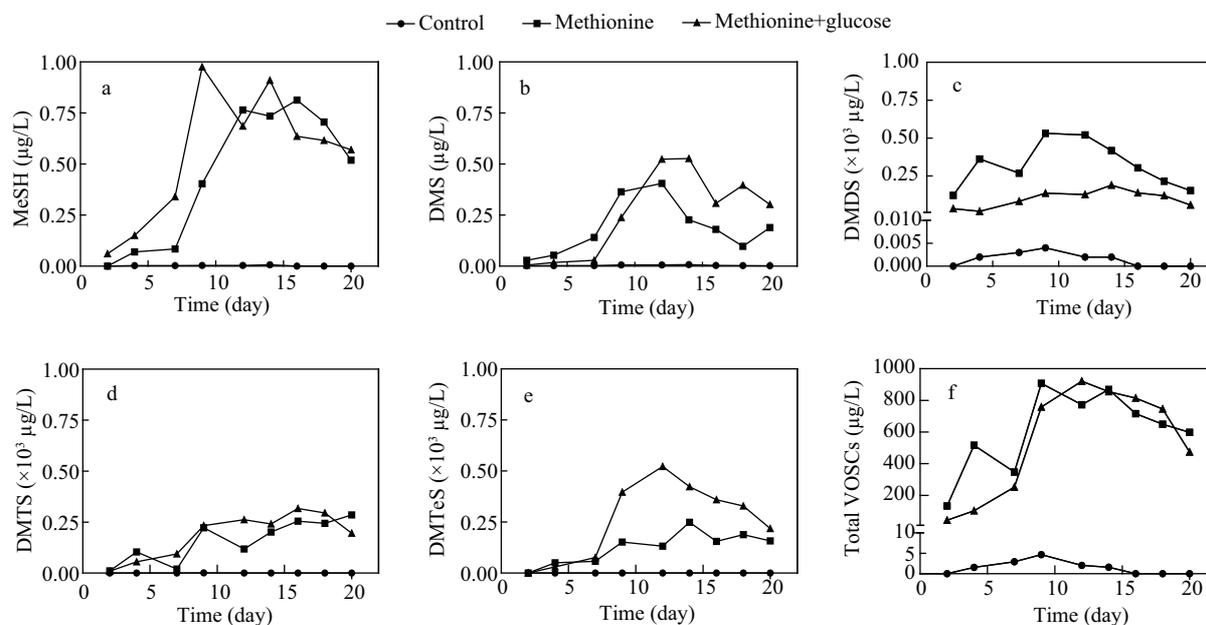


Fig. 6 Typical gas chromatogram of VOSC species obtained from water samples during the metabolism of methionine in this study.



**Fig. 7** Dynamics of five VOSC species: (a) MeSH, (b) DMS, (c) DMDS, (d) DMTS and (e) DMTeS as well as (f) total VOSCs produced over time in different treatments. Points represent the mean of two replicates. Standard errors were less than 10% of the means and are not shown.

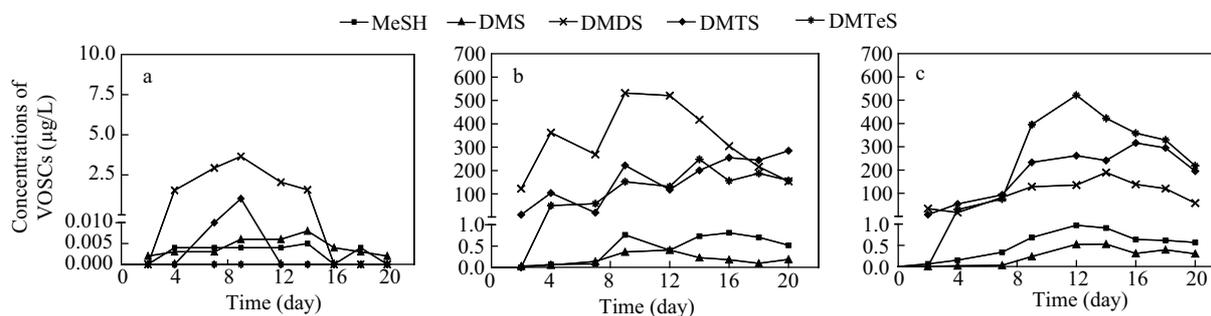
For the untreated control, all of the VOSCs except DMTeS were found in the samples (Fig. 8a). With the exception of the DMTS concentration on day 9, MeSH, DMS and DMDS were detected at relatively low concentrations of approximately 0–10 ng/L in water samples. In comparison, the concentration of DMDS was much higher with a peak value of 3.6  $\mu\text{g/L}$  on day 9. For the methionine alone treatment, DMDS was the most abundant species among the five VOSCs detected with a peak value of 532.2  $\mu\text{g/L}$ , followed by DMTS and DMTeS. MeSH and DMS were present in minor concentrations and together composed less than 0.1% of the total VOSCs released (Fig. 8b). DMTeS was the most important compound in the methionine+glucose treatment with a peak value of 522.1  $\mu\text{g/L}$ , followed by DMTS and DMDS, which was different from results in the methionine alone treatment. MeSH and DMS were minor and accounted for less than 0.15% of the total VOSCs released (Fig. 8c).

The total level of VOSCs generated from the untreated control was relatively low compared with those from the two methionine-amended treatments (Fig. 7f). In the

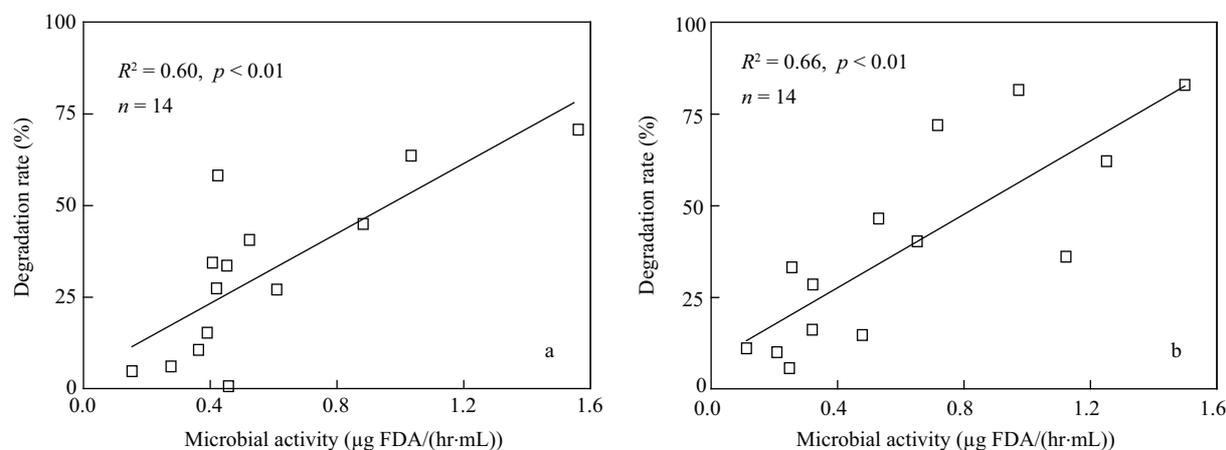
first 9 days, the total amount of VOSCs produced from methionine alone was higher than that produced from methionine+glucose. The situation changed after 3 days; total amounts of VOSCs from methionine+glucose increased rapidly and even exceeded those from methionine alone. Then, the total VOSCs decreased gradually after reaching a peak value (907.4  $\mu\text{g/L}$  on day 9 for methionine alone and 921.3  $\mu\text{g/L}$  on day 12 for methionine+glucose). Despite differences in the VOSC production patterns of the treatments, the amounts of total VOSCs produced in the experiment after additions of methionine alone and methionine+glucose were not significantly different overall).

### 3 Discussion

The experimental conditions for this study were strictly airtight to simulate the development of anaerobic processes through the action of the organic burden, the occurrence of black bloom and the production of VOSCs simultaneously.



**Fig. 8** Variations of MeSH, DMS, DMDS, DMTS and DMTeS in the treatments of control (a), methionine alone (b) and methionine+glucose (c). Points represent the mean of two replicates.



**Fig. 9** Correlation of microbial activity and the methionine degradation rate for methionine alone (a) and methionine+glucose (b) treatment.

The fact that methionine was found in the untreated control at the beginning of the incubation illustrates that methionine and its substrates may be common substances in eutrophic freshwater lakes that are affected by blue-green algal blooms. The substrates could be readily reduced to methionine and further decomposed under favorable conditions so that methionine increased in the first 3 days and was depleted quickly and completely in the following 2 days. It was observed that the production of VOSCs was considerable although a black color never occurred. The degradation rate of methionine in the methionine+glucose treatment was greater than that of methionine alone (Fig. 5), suggesting that the presence of glucose could stimulate the catabolism of methionine. The presence of glucose also led to higher microbial activity than methionine alone. The decomposition rate seemed to correspond to variations in microbial activity; statistical analysis showed that there were significant correlations between them (for methionine alone,  $R = 0.60$ ,  $p < 0.01$ ,  $n = 14$ ; for methionine + glucose,  $R = 0.66$ ,  $p < 0.01$ ,  $n = 14$ ) (Fig. 9). The results illustrate the involvement of environmental microorganisms in the metabolism of methionine.

We found that VOSCs were produced right from the beginning of the incubation for all of the treatments. However, the water began to turn black on day 9 and 12 and reached a climax on day 14 and 20 for the methionine alone and methionine+glucose treatments, respectively. Although existing knowledge indicates that the occurrence of the black color is always accompanied by the production of VOSCs, our present study indicated noncoincident production of VOSCs and the formation of a black color, but the coincidence of the total VOSC maximum with black color emergence implies the existence of a relationship between them. As the decomposition of organic matter could exhaust all dissolved oxygen rapidly and make the system extremely anaerobic, the water-sediment interface soon formed conditions of severe anoxia, and the Fe(II) precipitation-solution equilibrium was broken to render Fe-Mn oxides and sulfides deoxidized and eventually released into the strong reducing environment (Stahl, 1979;

Duval and Ludlam, 2001). In general, the concentration of  $\text{Fe}^{2+}$  and  $\text{Mn}^{2+}$  in the sediment-water interface peaked within several days of the organic burden application (Liu et al., 2009, 2010). However, methionine might be first turned into VOSCs through decomposition and further broken down to hydrogen sulfide. When the VOSCs accumulated abundantly, hydrogen sulfide might have been gradually produced and combined with available  $\text{Fe}^{2+}$ ,  $\text{Mn}^{2+}$  to form the black substance. This conceptualization does not preclude other parallel formation mechanisms such as the function of sulfate-reducing bacteria in the reduction of  $\text{SO}_4^{2-}$  to  $\text{S}^{2-}$  in an environment of extreme anoxia with abundant  $\text{SO}_4^{2-}$  (Jørgensen et al., 2001). The formation of metal sulfides darkened the whole system until available heavy metal ions were completely depleted. Therefore, the abundant production of VOSCs might be used as an important indicator of the outburst of black bloom. It is worth mentioning that after the chromaticity reached a peak, the black color spontaneously faded away in these static closed systems. It is widely believed that sediment particles are indispensable in the formation of black bloom. With sediment particles resuspended into overlying water under the influence of external forces, the produced FeS and MnS were chemically bound onto grain surfaces to form black spots that blackened the water (Lu and Ma, 2009; Kong et al., 2007). Once the external force retreated or the whole system was re-oxygenated, the black spots gradually precipitated or oxidized to clarify the water. Lacking an external disturbance and gas exchange, our reaction system was only influenced by the weak internal disturbance of rising gas bubbles. However, the blackening of the water could also appear and then spontaneously disappear. Accordingly, we speculated that sediment particles are not the fundamental cause of blackening; heavy metals ions, mainly  $\text{Fe}^{2+}$  and  $\text{Mn}^{2+}$  released from the sediment under anaerobic conditions contributed to the black color rather than the sediment particles themselves. Reduced sulfur produced during metabolic processes combined with available  $\text{Fe}^{2+}$  and  $\text{Mn}^{2+}$  in the water column to form black metal sulfides, which dispersed in the water column

until an abundance of them accumulated, flocculated and deposited to purify the water.

As observed in the present study, the reduced potentials that decreased slightly with the appearance black bloom never occurred in the untreated control. Compared with the methionine alone treatment, the Eh value for methionine+glucose decreased significantly, and abundant gases were released at an early stage (**Table 1, Fig. 3**). Surprisingly, the lower Eh did not result in an earlier occurrence of blackening. On the contrary, the water and sediment in the methionine alone treatment blackened earlier than those in the methionine+glucose treatment. Additional glucose decreased the anaerobic condition to a lesser degree and increased the production of gaseous compounds for a heavier organic burden (Devai and Delaune, 1995), but it inhibited the formation of black bloom.

Overall, the methionine+glucose treatment showed a higher microbial activity than the methionine alone treatment (**Fig. 4**), implying that additional glucose changed the overall metabolic rates as an additional carbon source. Glucose did accelerate the degradation rate of methionine, especially after day 10. However, the addition of glucose reduced total VOSC concentrations in the first 10 days (**Fig. 7f**) and delayed the formation of a black substance (**Table 1**), suggesting that the existence of glucose may change the transformation pathway of methionine and thereby inhibit the formation of VOSCs. Previous studies have found that the addition of acetate or its metabolic precursors (glucose) inhibited the thiol methylation potential that is vital for the formation and accumulation of VOSCs but did not inhibit CO<sub>2</sub> or CH<sub>4</sub> production (Finster et al., 1990). Working with peat from Sallies Fen in New Hampshire, Kiene and Hines (1995) found that high concentrations of glucose did not significantly change the overall metabolic rates but appeared to enhance CO<sub>2</sub> or CH<sub>4</sub> production and inhibit the thiol methylation potential and ultimately DMS formation. However, the inhibition of VOSCs production with glucose was significant only for the first 7 days of the incubation (**Fig. 7f**). Stets et al. (2004) also found that glucose-amended treatments had significantly greater rates of methane production for the first 91 hr of incubation. CO<sub>2</sub> concentrations also increased remarkably over the first 40 hr, but by the end of the incubation, CO<sub>2</sub> concentrations were not significantly different ( $P = 0.45$ , two-tailed *t*-test) in samples amended with glucose relative to untreated controls. In this study, the analysis of gas samples showed that CH<sub>4</sub> and CO<sub>2</sub> were predominant components in gas samples before day 5, amounting to  $3.5 \times 10^4$  and  $2.0 \times 10^5$  μL/L, respectively (data not shown). The abundant gases that evolved from methionine+glucose were CH<sub>4</sub> and CO<sub>2</sub> in this stage. After day 9, with gas regenerated, the concentrations of CO<sub>2</sub> and CH<sub>4</sub> were significantly reduced, while VOSCs, mainly MeSH, DMS and DMDS, were released in high amounts with concentration increases from 6.4, 0.14 and 0.19 μL/L

to  $1.1 \times 10^4$ , 176.90 and 7.81 μL/L, respectively, from day 9 to day 20. In the methionine alone treatment, no gas was produced before day 14. After that, the gas was collected and its constituents found to be similar to those of the methionine+glucose treatment. These results indicate that with the inhibitory effect of glucose eventually relieved, metabolism may primarily proceed toward the production of VOSCs when abundant VOSCs are produced and volatilized into the gas phase.

For specific VOSCs species, MeSH in water samples was below detection limits or at low concentrations at the beginning and then accumulated slightly over time. A possible explanation for the misdetection or low detection is that MeSH has been found to be a precursor of structurally diverse sulfur compounds such as DMDS and DMTS, and it is unstable in aqueous solutions (Bloes-Breton and Bergre, 1997; Bonnarne, 2000). Chin and Lindsay (1994) found that MeSH can be readily oxidized by non-enzymatic auto-oxidation in an aqueous solution to form other VOSCs including DMDS, DMTS and DMTeS. Therefore, at the beginning of decomposition, MeSH is transformed into dimethyl polysulfide and does not accumulate much until an appreciable amount is produced. In addition, the poor water solubility and high volatility of MeSH may be responsible for its low detection in water samples. The analysis of gas samples showed high MeSH concentrations of up to  $1.1 \times 10^4$  μL/L. Despite the total VOSCs in methionine+glucose being less than that in methionine alone, MeSH in methionine+glucose was present in higher concentrations before day 10. The function of Eh on MeSH oxidation might be an important reason. As the Eh value for methionine+glucose was significantly lower than that for methionine alone before day 10, MeSH in the methionine+glucose treatment was not more easily oxidized than that in the methionine alone treatment. With the Eh value increasing after day 5 and reaching values even higher than those for methionine alone after day 10, MeSH was more readily converted into dimethyl polysulfides and less accumulated, which led to a considerable increase in total VOSCs in the methionine+glucose treatment after day 5. The DMS concentration in aqueous samples was relatively low compared with other dimethyl polysulfide forms. Two potential explanations exist. Similarly to MeSH, DMS shows poor water solubility and high volatility, and was found at 176.90 μL/L in gaseous samples. Alternatively, DMS is probably generated by a secondary mechanism that is distinct from that which forms other dimethyl polysulfides (Arfi et al., 2002; Demarigny et al., 2000). Other research also indicates that DMS follows a less important production pattern than that of MeSH and other sulfides, so DMS was produced in lesser amounts than other dimethyl polysulfide species (Bonnarne et al., 2001).

DMDS was found to be the most abundant species in water with a maximum concentration of 3.6 μg/L on day

9 in the untreated control, implying that DMDS might be the primary VOSC species produced in the metabolism of methionine at low concentrations. The amount of DMDS and its predominance were similar to the results of previous studies on VOSCs in natural aquatic systems (Gun et al., 2000). The observation of considerable DMDS in the untreated control indicates that the taste and odor of VOSCs are very common in eutrophic lakes. However, only when the organic burden is high enough are the odor and black color produced to form black water agglomerate. DMDS, DMTS and DMTeS were the dominant polysulfide forms in the two methionine-amended treatments. Although the two treatments produced a similar amount of total VOSCs by the end of the study, the composition and concentration of VOSC species were markedly different. For the methionine alone treatment, the lower molecular VOSC species DMDS was found to be principally in organic dimethyl polysulfide forms rather than in larger molecules such as DMTeS and DMTS. By comparison, larger molecular weight DMTeS was more abundant than DMTS and DMDS in the methionine+glucose treatment, suggesting that glucose tends to extend disulfide bonds to form larger molecular weight compounds. Therefore, for the methionine+glucose treatment, simple-structured VOSC species and hydrogen sulfide were produced in smaller amounts, resulting in the retardation of black bloom formation compared with the methionine alone treatment.

## 4 Conclusions

The taste and odor of VOSCs are commonly found in eutrophic lake water suffering from algal blooms. However, only when methionine was added were both foul odor and black substance produced to form black bloom. There was a progressive and significant increase in the five VOSC species studied with applied methionine, suggesting that methionine acts as an important precursor in the production of VOSCs in lakes suffering from algal blooms and also algae-induced black bloom. The low Eh value of the system provided favorable conditions for heavy metals in sediments to be released to the overlying water to combine with  $S^{2-}$  to form black substances. Microorganisms play an important role in the metabolism of methionine. To clarify the production mechanism of VOSCs in black bloom, it is essential to isolate, incubate and cultivate the microorganism(s) responsible for methionine degradation in further studies.

The VOSC production was observed to precede black color development, and the appearance of a VOSC maximum was often followed by water blackening. Additional glucose accelerated the degradation of methionine, postponed the occurrence of black bloom and inhibited the transformation of methionine into VOSCs, especially in the first 7 days. With the inhibitory effect of glucose

eventually relieved, metabolism may primarily proceed toward the later production of VOSCs, so that the total amount of VOSCs appeared to be almost identical at the end for the two amended treatments. DMDS was found to be the most abundant species for the methionine alone treatment, and DMTeS seemed to be the most important compound in the methionine+glucose treatment, implying that the existence of glucose may change the transformation pathway of methionine into VOSCs to form the larger molecular weight compounds DMTS and DMTeS. Only organic sulfur compounds were measured in the present study, and hydrogen sulfide was not included in our research. Hydrogen sulfide is an important form of reduced sulfur that is produced in the formation of the black substance that has been detected during the decomposition of sulfur-containing organic matter in previous studies (e.g., Kim et al., 2009), and it should be considered in future studies.

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