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CONTENTS

Aquatic environment

- Effect of Na⁺ impregnated activated carbon on the adsorption of NH₄⁺-N from aqueous solution
Mo Shi, Zhengfang Wang, Zheng Zheng 1501
- Fate and degradation of nonylphenolic compounds during wastewater treatment process
Jing Lian, Junxin Liu 1511
- Comparison of UV/PDS and UV/H₂O₂ processes for the degradation of atenolol in water
Xiaowei Liu, Lei Fang, Yongchao Zhou, Tuqiao Zhang, Yu Shao 1519
- Biodegradation of alkylates under less agitated aquifer conditions
Jay J. Cho, Makram T. Suidan, Albert D. Venosa 1529
- Characterization of typical taste and odor compounds formed by *Microcystis aeruginosa*
Kejia Zhang, Tsair Fuh Lin, Tuqiao Zhang, Cong Li, Naiyun Gao 1539
- Application of high OLR-fed aerobic granules for the treatment of low-strength wastewater: Performance, granule morphology and microbial community
Jingyun Ma, Xiangchun Quan, Huai Li 1549
- Optimization and evaluation of a bottom substrate denitrification tank for nitrate removal from a recirculating aquaculture system
Wiboonluk Pungrasmi, Cholticha Playchoom, Sorawit Powtongsook 1557
- Coking wastewater treatment for industrial reuse purpose: Combining biological processes with ultrafiltration, nanofiltration and reverse osmosis
Xuewen Jin, Enchao Li, Shuguang Lu, Zhaofu Qiu, Qian Sui 1565
- Interference of iron as a coagulant on MIB removal by powdered activated carbon adsorption for low turbidity waters
Ferreira Filho SIDNEY SECKLER, Marchetto MARGARIDA, Alves Laganaro ROSEMEIRE 1575
- Supercritical water oxidation of polyvinyl alcohol and desizing wastewater: Influence of NaOH on the organic decomposition
Jie Zhang, Shuzhong Wang, Yang Guo, Donghai Xu, Yanmeng Gong, Xingying Tang 1583
- Investigation of potential interferences on the measurement of dissolved reactive phosphate using zirconium oxide-based DGT technique
Qin Sun, Yifei Chen, Di Xu, Yan Wang, Shiming Ding 1592

Atmospheric environment

- Disturbance of light-absorbing aerosols on the albedo in a winter snowpack of Central Tibet
Jing Ming, Pengling Wang, Shuyu Zhao, Pengfei Chen 1601
- Application of AERMOD on near future air quality simulation under the latest national emission control policy of China: A case study on an industrial city
Jieyun Ma, Honghong Yi, Xiaolong Tang, Yan Zhang, Ying Xiang, Li Pu 1608
- High black carbon and ozone concentrations during pollution transport in the Himalayas: Five years of continuous observations at NCO-P global GAWstation
A. Marinoni, P. Cristofanelli, P. Laj, R. Duchi, D. Putero, F. Calzolari, T. C. Landi, E. Vuillermoz, M. Maione, P. Bonasoni .. 1618
- Chemical composition, mass closure and sources of atmospheric PM₁₀ from industrial sites in Shenzhen, China
Gang Wu, Xin Du, Xuefang Wu, Xiao Fu, Shaofei Kong, Jianhua Chen, Zongshuang Wang, Zhipeng Bai 1626

Terrestrial environment

- Spatial distribution of polychlorinated biphenyls in soil around a municipal solid waste incinerator
Wenbin Liu, Haifeng Li, Zhenyu Tian, Huiting Xie, Jicheng Hu 1636

Multi-factors influencing the spatial distribution of polycyclic aromatic hydrocarbons in soils surrounding drinking water protection zone	
Wentao Jiao, Tieyu Wang, Yonglong Lu, Andrew Chang, Weiping Chen	1643

Environmental biology

Denaturing gradient gel electrophoresis fingerprinting of soil bacteria in the vicinity of the Chinese GreatWall Station, King George Island, Antarctica (Cover story)	
Qi Pan, Feng Wang, Yang Zhang, Minghong Cai, Jianfeng He, Haizhen Yang	1649
A preliminary investigation on the occurrence and distribution of antibiotic resistance genes in the Beijiang River, South China	
Zhenhao Ling, Ying Yang, Yuanli Huang, Shichun Zou, Tiangang Luan	1656

Environmental health and toxicology

Toxicity of the xenoestrogen nonylphenol and its biodegradation by the alga <i>Cyclotella caspia</i>	
Yu Liu, Xiaokang Dai, Jie Wei	1662
Ecotoxicological effects of waterborne PFOS exposure on swimming performance and energy expenditure in juvenile goldfish (<i>Carassius auratus</i>)	
Jigang Xia, Shijian Fu, Zhendong Cao, Jianglan Peng, Jing Peng, Tingting Dai, Lili Cheng	1672

Environmental catalysis and materials

Photocatalytic degradation of carbofuran in TiO ₂ aqueous solution: Kinetics using design of experiments and mechanism by HPLC/MS/MS	
Hai Yang, Shuolin Zhou, Huajie Liu, Weiwei Yan, Liping Yang, Bing Yi	1680
Mesopolymer modified with palladium phthalocyaninesulfonate as a versatile photocatalyst for phenol and bisphenol A degradation under visible light irradiation	
Rong Xing, Lin Wu, Zhenghao Fei, Peng Wu	1687
Formic acid as an alternative reducing agent for the catalytic nitrate reduction in aqueous media	
Eun-kyoung Choi, Kuy-hyun Park, Ho-bin Lee, Misun Cho, Samyoung Ahn	1696
Promotional effect of Si-doped V ₂ O ₅ /TiO ₂ for selective catalytic reduction of NO _x by NH ₃	
Yanxiao Pan, Wei Zhao, Qin Zhong, Wei Cai, Hongyu Li	1703

Environmental analytical methods

Determination of 4-tert-octylphenol in surface water samples of Jinan in China by solid phase extraction coupled with GC-MS	
Xueyu Yang, Mingren Liu, Zhongpeng Wang, Qian Li, Zhaoliang Zhang	1712

Serial parameter: CN 11-2629/X*1989*m*217*en*P*26*2013-8



Characterization of typical taste and odor compounds formed by *Microcystis aeruginosa*

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Abstract

Production and characteristics of typical taste and odor (T&O) compounds by *Microcystis aeruginosa* were investigated. A few terpenoid chemicals, including 2-MIB, β -cyclocitral, and β -ionone, and a few sulfur compounds, such as dimethyl sulfide and dimethyl trisulfide, were detected. β -Cyclocitral and β -carotene concentrations were observed to be relevant to the growth phases of *Microcystis*. During the stable growth phase, 41–865 fg/cell of β -cyclocitral were found in the laboratory culture. β -Cyclocitral concentrations correlated closely with β -carotene concentrations, with the correlation coefficient $R^2 = 0.96$, as it is formed from the cleavage reaction of β -carotene. For dead cell cases, a high concentration of dimethyl trisulfide was detected at 3.48–6.37 fg/cell. Four T&O compounds, including β -cyclocitral, β -ionone, heptanal and dimethyl trisulfide, were tested and found to be able to inhibit and damage *Microcystis* cells to varying degrees. Among these chemicals, β -cyclocitral has the strongest ability to quickly rupture cells.

Key words: *Microcystis aeruginosa*; T&O compounds; β -cyclocitral; algae-lytic effect

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Introduction

In the past few decades, increasing concentrations of nutrients have been observed in many rivers, lakes, and reservoirs around the world, leading to frequent outbreaks of cyanobacterial blooms (Li et al., 2012). The major water quality implication of the proliferation of cyanobacterial blooms is the production of secondary metabolites (Zhou et al., 2012). In particular, metabolites which can cause aesthetic issues, such as taste and odor (T&O) compounds, and those which severely impact human health, such as cyanobacterial toxins, are of concern in drinking water systems.

Although in many cases T&O compounds do not pose a threat to human health, they may affect the public's perception on the safety of drinking water (Peter and von Gunten, 2007; Peter et al., 2009), and this can lead to a lack of trust in the water industry. The odor types commonly observed in drinking water are categorized into eight groups, as described in the water flavor wheel (Suffet et al., 1999). Among the eight odor groups, many studies have focused on the earthy/musty odor type, with two

cyanobacteria associated metabolites, geosmin and 2-MIB, being highlighted as the typical odorants responsible for this. Besides geosmin and 2-MIB, β -cyclocitral, β -ionone, heptanal, dimethyl sulfide and dimethyl trisulfide are also frequently encountered odorants during cyanobacteria blooms (Deng et al., 2012; Ginzburg et al., 1998; Yang et al., 2008). Both β -cyclocitral and β -ionone are products of the oxidative decomposition of β -carotene present in *Microcystis* (Jüttner and Hoflacher, 1985). β -cyclocitral has a sweet-tobacco and grape odor (Zimba and Crimm, 2003), and has been found at trace levels of 500 ng/L in natural water (Young et al., 1999) and at cell quota of 15 ng/10⁶ cells in *M. aeruginosa* culture (Dietrich et al., 1995). For β -ionone, the odor threshold is only 7 ng/L (Jones and Korth, 1995), with flowery and violets odor. Two sulfur related chemicals, dimethyl sulfide and dimethyl trisulfide, are important T&O-causing compounds in China, mainly responsible for the septic/swampy odor (Yu et al., 2009), and these have also been identified as common odor-producing compounds by *Microcystis*, particularly during the decaying stage (Jüttner, 1984).

Microcystis aeruginosa is one of the most common cyanobacteria found in fresh water blooms (Zhang et al., 2010), and has been studied extensively with regard to its

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physiology and ecology. Because of its ability to produce microcystins, management and treatment of *M. aeruginosa* and the related microcystins in drinking water have also been well documented (Ho et al., 2011; Teixeira and Rosa, 2007; Wu et al., 2012). Most studies have mainly focused on toxin-related issues for specific cyanobacterium, and few have examined the T&O compounds associated with this species. The production of typical T&O compounds and their patterns for *M. aeruginosa* are thus still unclear. Therefore, the major objectives of this study are to detect the production of major T&O compounds produced by *M. aeruginosa*, examine the correlations among the T&O compounds, and understand their production patterns during different growth phases.

1 Materials and methods

1.1 Chemicals and cell culture

The standards of the target analytes (β -cyclocitral, β -ionone, dimethyl sulfide, dimethyl trisulfide and heptanal) were purchased from Sigma-Aldrich (USA). The internal standard 2-isobutyl-3-methoxy pyrazine (IB, 99%) was purchased from Fluka (Japan). Analytical grade NaCl was obtained from Sinopharm Chemical Reagent Co., China, and heated to 450°C for 2 hr before use.

Microcystis aeruginosa (905), purchased from the Institute of Hydrobiology, Chinese Academy of Science, was cultured in BG11 medium in an incubator at 25°C under a constant light flux (2000 lux) with a light/dark cycle of 12 hr/12 hr.

1.2 Experimental procedures

1.2.1 Determination of β -cyclocitral and β -carotene of *M. aeruginosa*

Bloom samples were collected weekly during Jul 25 to Sep 8, 2010, from Lake Taihu, China. All the samples from 0.5 m below the surface were collected at the same site of Meiliang Bay. A commercially available fiber (30/50 μ m DVB/CAR/PDMS (57328-U) and a manual fiber holder (57330-U), both from Supelco (Bellefonte, PA, USA), were used for the extraction of the odorants. Major volatile and semi-volatile organic compounds were determined using a gas chromatograph and mass spectrometer (GC-MS, QP2010S, Shimadzu, Japan) in the scanning mode.

Algal solution of $6.1 \times 10^7 \pm 4.3 \times 10^6$ cells/L *M. aeruginosa* was cultured in BG11 for more than 50 days, through adaptive, log, stable and decayed phases. At predetermined time intervals (day 1, 2, 6, 9, 15, 20, 24, 28, 35, 40, 47 and 50), the algal solution was sampled for cell counting and T&O compound analysis. For cell counting, an aliquot (1 mL) of the sample was transferred to a 1 mL custom counting chamber and analyzed with a microscope (BX51, Olympus, Japan) at a magnification of 400 times. For the analysis of T&O compounds, a 50 mL sample was first

filtered through a 0.50- μ m glass filter (90 mm i.d., Whatman, England) to separate cell-bound and extracellular components. Both the filtrate and unfiltered sample were analyzed for the targeted T&O compounds. In addition, the precursors of β -cyclocitral, β -carotene, were also analyzed. Before the analysis, β -carotene particles in water were first collected with centrifugal filter devices (0.22 μ m) (Ultrafree-MC, Millipore) and then dissolved with furan (GR grade, Fluka, Japan).

1.2.2 T&O compounds formed after *M. aeruginosa* decayed

The algal solution was harvested during its stable phase (the density of $4.0 \times 10^8 \pm 3.8 \times 10^7$ cells/L), and then subjected to three freeze/thawing cycles to accelerate the breaking of the cells. Some of the cyanobacteria then continued to be cultured in the incubator at 25°C, and others were cultured under anaerobic conditions at 25 or 4°C. After 10 days, the cyanobacteria decayed to various degrees. The concentrations of T&O compounds in the samples were determined with the SPME-GC/MS method.

1.2.3 Lysis of *M. aeruginosa* cells using T&O compounds

Several T&O compounds have been reported to have ability of lysing cyanobacteria and algae (Chang et al., 2011; Ozaki et al., 2008). In this study, the effects of typical T&O compounds on cell integrity were also examined. Briefly, 200 μ L of pure T&O compound chemicals (dimethyl sulfide, dimethyl trisulfide, β -cyclocitral, β -ionone and heptanal) were added to 200 mL of the cultured *M. aeruginosa* (the density of $4.7 \times 10^9 \pm 6.1 \times 10^8$ cells/L), and incubated at 25°C for 4 days. At designed time intervals (1, 3, 8, 18, 32, 44 and 92 hr), the color changes of algae solution were recorded using a 12 million pixel digital camera (TX1, Sony, Japan). In addition, 5 mL of the cultured sample was taken to determine the activity of the cells.

1.3 Analysis

The selected T&O compounds, including dimethyl trisulfide, β -cyclocitral and β -ionone and internal standard IB, were quantified using SPME coupled with GC-MS, using the analysis procedure reported in our previous studies (Zhang et al., 2011). In the analysis, selected ion monitoring mode was chosen with m/z of 126 and 79 for dimethyl trisulfide, m/z of 137 and 152 for β -cyclocitral, and m/z of 177 and 135 for β -ionone, and m/z of 124 and 94 for IB, respectively. β -Carotene analysis was carried out using a liquid chromatograph equipped with two identical pumps (LC-10AD, Shimadzu, Japan), a reverse phase C18 analytical column (100 mm \times 4.6 mm i.d.) (Chromolith Performace RP-18e, Merck, Germany), and a mass spectrometer (2010EV, Shimadzu, Japan). Detailed procedures for this analysis are available in previous publication (Zhang et al., 2011).

Two parameters, optical density at 680 nm (OD_{680}) and photosynthetic efficiency, were used as indexes for *M. aeruginosa* cell count and cell activity, respectively (Ou et al., 2011). In this study, the OD_{680} value was determined using a UV1800 ultraviolet/visual spectrophotometer (UV2802, UNICO, USA). Photosynthetic efficiency represents the maximum rate of increase for light-limited photosynthesis by a unit of $\mu\text{mol electrons m}^{-2}\text{sec}^{-1}/\mu\text{mol photons m}^{-2}\text{sec}^{-1}$, and this was determined with a phytoplankton analyzer (PHYTO-PAM, Walz, Germany). Another parameter, dissolved oxygen (DO) was measured with a DO meter (LDOTM, Hach, USA).

2 Results and discussion

2.1 Volatile metabolites of *Microcystis* bloom

SPME and GC/MS analysis were used to detect volatile and semi-volatile compounds from the water samples containing cyanobacteria (Table 1). Twelve metabolites could be simultaneously identified using scanning mode and similarity analysis. Of these, dimethyl trisulfide, heptanal, β -cyclocitral and β -ionone were found to be the dominant T&O compounds, and are studied in more detail in the following sections.

2.2 *Microcystis* and β -cyclocitral

Figure 1 shows that the cell concentrations of *M. aeruginosa* remained constant for the first two days, implying the adaptation of the strain to the new environment. After two days, the cells were in the log growth phase until day 24, with the cell number increasing from 6.1×10^7 to 3.6×10^9 cells/L. In the second half of the experimental period, the number of cells began fluctuated in the range of 1.5×10^9 to 2.5×10^9 cells/L, showing that the strain was in the stable growth phase.

Figure 1 also shows the total and extracellular concentrations of β -cyclocitral in the cyanobacteria culture

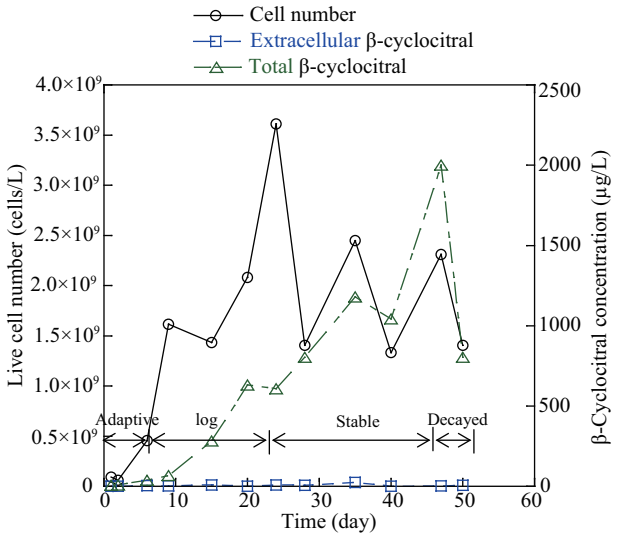


Fig. 1 Time course of cell numbers and β -cyclocitral concentrations for the growth of *M. aeruginosa*.

solution sampled at the same time. During the experimental period, the extracellular portions of β -cyclocitral were always less than 1/100 of the total concentrations, indicating that most of the β -cyclocitral is within the cells. As β -cyclocitral is known to be produced by the cleavage of β -carotene after cells are ruptured (Jüttner and Hoflacher, 1985), it is expected to be produced during the analytical processes, in which the high concentrations of NaCl used to enhance the SPME pre-concentration of the T&O compounds may damage the cells (Zhang et al., 2012). Therefore, it is reasonable to assume that the *M. aeruginosa* cells in the present experiment were mostly undamaged. In natural waters, when *M. aeruginosa* is in the log and stable growth phases and the cells are mostly healthy, β -cyclocitral is expected to be in a very low concentration in the water, and thus no β -cyclocitral associated off-flavor problems are expected. However, if the cells begin to decay or are damaged by oxidation in typical water treatment processes, β -cyclocitral is expected to be produced and released into the water, causing woody or tobacco odors. Jones and Korth (1995) reported that the average cell quota of β -cyclocitral for *M. aeruginosa* is 10 fg/cell. However, in the present study, a much higher cell quota, in the range of 41–865 fg/cell, was observed, and there are several reasons that may explain this. In the study by Jones and Korth (1995), the estimation is based on field samples with a *Microcystis* assemblage, as opposed to the single *M. aeruginosa* strain grown under excess nutrient conditions examined in this study. In addition, as β -cyclocitral is known to be produced mostly during analysis procedures, different experimental methods may cause different degrees of β -cyclocitral production (Ikawa et al., 2001).

Since β -cyclocitral is an exclusive metabolite of *Microcystis*, the correlation between cell number and total

Table 1 Volatile and semi-volatile organic compounds from *Microcystis* bloom

Number	Organic compounds	Similarity (%)	Response time (min)
1	1-Hexanol	96	5.208
2	Benzocyclobutene	91	5.625
3	Dimethyl trisulfide	97	5.732
4	Heptanal	97	5.810
5	(Z)-2-Gene aldehyde	96	6.891
6	2-Octene aldehyde	95	8.900
7	4-Methylphenol	97	9.499
8	Nonanal	97	9.784
9	β -Cyclocitral	95	12.208
10	(E,E)-2,4-sebacic ene aldehyde	96	13.850
11	β -Ionone	94	16.542
12	2,4-Tert-butylphenol	95	16.875

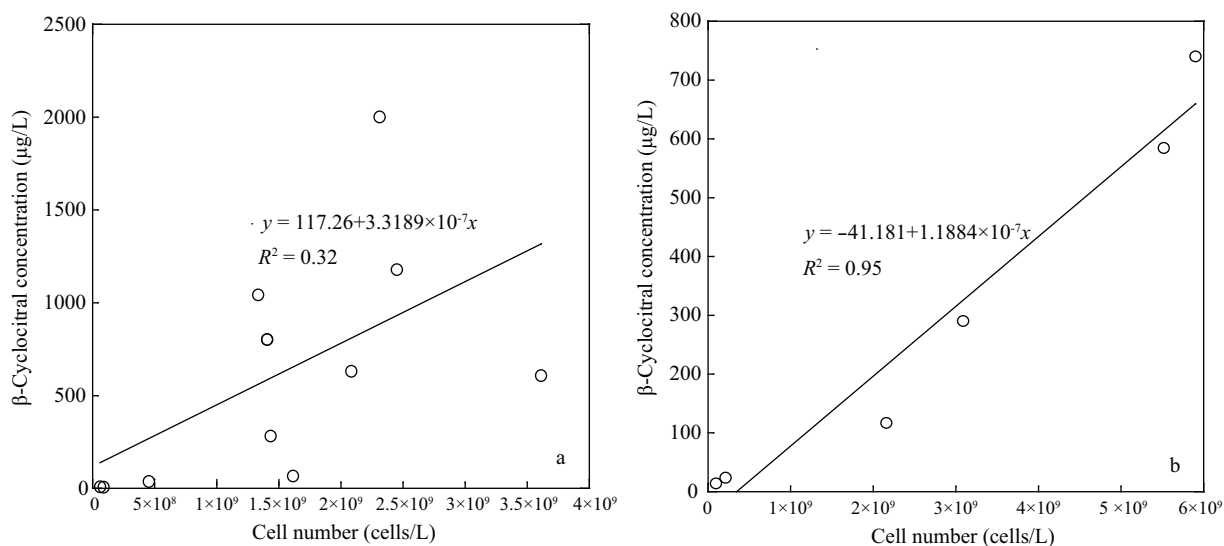


Fig. 2 Correlations between cell numbers and β-cyclocitral concentrations. (a) cultured in laboratory; (b) in Lake Taihu.

β-cyclocitral concentration is shown in Fig. 2a. Surprisingly, no strong correlation is observed for the samples collected from the adaption period to declination period, because R^2 is only 0.32. Therefore, the concentration of β-cyclocitral may not only be associated with the amount of algae cells, but also with the growth phase of *Microcystis*. In the previous reports, some unfavorable factors affected the growth and photosystem II activity of *M. aeruginosa*, causing the concentration of chlorophyll *a* and carotene decreased (Ou et al., 2005; Wang et al., 2012). However, this is the first time to illustrate the correlation between β-cyclocitral and the growth status of *Microcystis*.

Samples were also collected from Lake Taihu in summer, and the data for the *Microcystis* cells and β-cyclocitral concentrations are plotted in Fig. 2b. The *Microcystis* produced β-cyclocitral 54–145 fg per cell, which are lower than the cell quota in the log and stable phases. That is because cyanobacteria are capable of producing photoprotective compounds and deactivating the carotenoids under high irradiance and the amount volatile organic compounds, eg. β-cyclocitral, β-ionone and so on, will be reduced (Walsh et al., 1998; Schagerl and Muller, 2006). As shown in Fig. 2b, excellent correlation was found for the *Microcystis* cells and β-cyclocitral concentrations in the Lake Taihu samples, with $R^2 = 0.95$. As all the water samples were collected from the same depth (0.5 m below water surface) during the *Microcystis* bloom period (July 25 to Sept 8, 2010), it is speculated that almost the *Microcystis* cells were in the same growth phase. Tan et al. (2009) also found little difference in *Microcystis* diversity in the same season by means of 16S-23S rRNA ITS analysis. Therefore, in field conditions, β-cyclocitral concentration may be used to estimate *Microcystis* cell concentrations in the water samples, if the cells are roughly in the same growth phase, eg. in the same season.

2.3 Relationship between β-carotene and β-cyclocitral

Although *Microcystis* cells produce a considerable amount of β-cyclocitral, cell concentrations and β-cyclocitral concentrations may be different from phase to phase, as discussed in Section 2.2. When cells are in the adaptive phase, the average cell quota of β-cyclocitral is only 41 fg/cell; when cells are in the log and stable phases, more β-cyclocitral is produced (the quota is 168–865 fg/cell). Many studies have demonstrated that β-cyclocitral is produced from β-carotene in the cells (Dietrich and Hoehn, 1995; Jüttner and Hoflacher, 1985; Sommerburg et al., 2003). In *Microcystis* cells, β-carotene is located in the cytoplasm, and β-carotene dioxygenase is on the cell membrane. Only after a cell is damaged will the β-carotene come into contact with the dioxygenase and oxygen, quickly triggering the chain scission reaction to produce β-cyclocitral (Sommerburg et al., 2003). Schagerl and Muller (2006) pointed out that carotenoids are present in cyanobacteria, and the amount of β-carotene, one of the carotenoid contents in cyanobacteria, varies considerably with growth conditions. As the cleavage effect of β-carotene and production of β-cyclocitral are unique for *Microcystis*, the correlation between β-carotene and β-cyclocitral at different growth phases is further analyzed.

Figure 3 shows the relationships between β-carotene and β-cyclocitral concentrations for the laboratory *Microcystis* culture. A strong correlation can be seen (with $R^2 = 0.96$), even though the samples were taken at different growth phases. Based on the slope of the best fitting curve, it may be inferred that the concentration of β-cyclocitral is 9% that of β-carotene. Considering the difference in molecular weight of the two compounds, the mole ratio (β-cyclocitral/β-carotene) is 0.32. Since the cleavage reaction of one β-carotene molecule may produce two molecules of β-cyclocitral, the transformation of β-carotene to β-

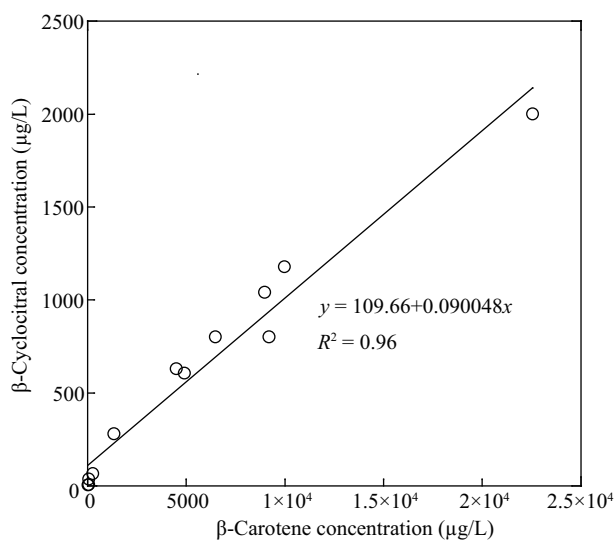


Fig. 3 Correlation between the concentrations of β -carotene and β -cyclocitral for laboratory grown *M. aeruginosa*.

cyclocitral in our experimental system is 0.16. Zhang et al. (2011) studied the chemical oxidation of β -carotene and β -cyclocitral using permanganate. They observed that the transformation of β -carotene to β -cyclocitral is 0.13, which is very similar to the finding of this study. As β -carotene is hardly dissolved in water and is present in small particle form, it is very likely that there is a mass transfer limitation for the oxidant as described by Zhang et al. (2011) and the oxygen and oxygenase in this study to be in contact with β -carotene, and thus transformation rates of 0.13 to 0.16 are observed.

As discussed previously, β -carotene is closely related to the growth phases of cyanobacteria (Schagerl and Muller, 2006), and correlates well with β -cyclocitral in the same samples. Therefore, it may provide an easy way to measure the growth state of *Microcystis*, since β -cyclocitral is one of the unique cleavage products of *Microcystis* (Fujise et al., 2010). When *Microcystis* cells are healthy, i.e. in the log phase, it is expected that more β -cyclocitral will be detected, as more β -carotene is available. However, if the algae cells are in the decayed phase, less β -cyclocitral will be detected. Since the measurement of β -cyclocitral, using GC/MS method, is usually simpler and more precise compared with that of β -carotene, typically using liquid chromatograph and requiring pre-treatment with solvent, this may provide a quick diagnostic method to examine the growth phase of *Microcystis*.

2.4 Odor chemicals produced from decayed *Microcystis*

Cyanobacteria associated odorants may be produced in two ways. The first is that they are produced in cells and then actively released into water when the cells are alive, or passively released when they are lysed. The second is that they are produced from bio-degradation of cells when cells have died (Wang and Gao, 1999). The latter process is explored in more detail. The frozen/thawed *M. aeruginosa* cells were incubated for 10 days, and the solution samples were analyzed using GC/MS for probable odor compounds.

Figure 4 shows the chromatograph spectrum of the live and dead *M. aeruginosa* cultured samples. It can be seen that the chromatograph peak at 6.75 min observed for the live cell sample disappeared for the dead one, although a new peak appeared at 5.75 min for the latter. The peak at 6.75 min was due to disulfide compounds, and the one at 5.75 min was due to dimethyl trisulfide. This suggests that dimethyl trisulfide is produced during the degradation of cyanobacterial cells. Dimethyl trisulfide is known to be derived from microbial decomposition of dimethyl sulfonic propionate. Once the cells are dead, the chemical may enter the water, and first be transferred dimethyl disulfide, and then dimethyl trisulfide (Jiang et al., 1997).

To quantitatively analyze the odor compound produced from dead *Microcystis* under different oxygen and temperature conditions, experiments were conducted for different treatments of algae solution, including an open bottle with oxygen exchange (Case B), and sealed bottles without oxygen exchange (Cases C and D). Note that Cases B and C were incubated at 25°C, while Case D was at 4°C. For comparison, live *M. aeruginosa* cells (Case A) were also grown under the same conditions as Case B. The samples were analyzed at day 10 for T&O compounds (Cases B, C and D). **Table 2** lists the concentrations of T&O compounds detected for the four cases. Comparing Cases B and A, both incubated under aerobic and 25°C conditions, the concentration of β -cyclocitral for Case B (the dead cell case) decreased by 27%, from 32420.00 to 23531.20 ng/L, while that of β -ionone increased two-fold, from 119.00 to 233.80 ng/L. It is possible that a portion of β -carotene oxygenase was damaged during the repeated freezing-thawing processes for cell treatment, causing a lower β -cyclocitral production rate. With regard to β -ionone, it forms more readily when β -carotene is degraded by filamentous fungi (Zorn et al., 2003). In the experimental system of Case B,

Table 2 Concentrations of odorants under different cultivation conditions (cell number: $4.0 \times 10^8 \pm 3.8 \times 10^7$ cells/L)

Condition	Cultivation		Dimethyl trisulfide (ng/L)	2-MIB (ng/L)	GSM (ng/L)	β -Cyclocitral (ng/L)	β -Ionone (ng/L)
	DO (mg/L)	Temperature (°C)					
A-live	6.1	25	26.90	308.00	24.00	32420.00	119.00
B-dead	5.4	25	25.80	363.00	22.16	23531.20	233.80
C-dead	0.34	25	2580.00	795.00	54.00	135.00	628.00
D-dead	0.55	4	1410.00	640.00	32.00	1780.00	1100.00

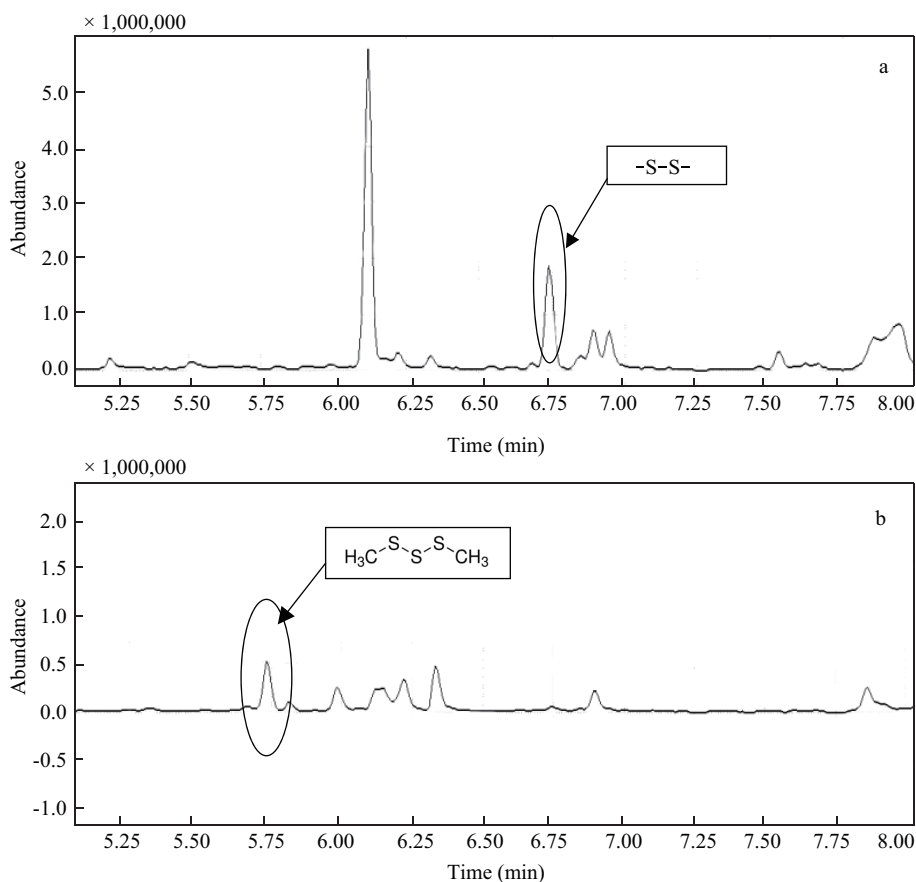


Fig. 4 Chromatograms from GC-MS analysis for the aqueous samples of live (a) and dead (b) *M. aeruginosa*. Cell number: $4.0 \times 10^8 \pm 3.8 \times 10^7$ cells/L; -S-S-: disulfide compounds; $\text{CH}_3\text{-S-S-S-CH}_3$: dimethyl trisulfide.

as the cells were dead, fungi may have been present in the samples, and this could have increased the formation of β -ionone.

Table 2 also shows that, under hypoxic conditions, dimethyl trisulfide concentrations increased significantly, from 26.90 to 2580.00 ng/L or 1410.00 ng/L, for Cases C (25°C) or D (4°C), respectively. The results suggest that dimethyl trisulfide formed under a more reduced environment, in which the DO concentration was low. For example, in May of 2007, as much as 11.39 ng/L dimethyl trisulfide was detected in raw water in Wuxi City, China (Yang et al., 2008), most likely caused by anaerobic decomposition of algae, according to our findings. In addition, 2-MIB and geosmin were also found to increase two-fold after the cells were ruptured. This may be attributed to the fact that actinomycetes rely on the cell debris to grow, and produce the two chemicals under hypoxic conditions (Srinivasan and Sorial, 2011). Unlike dimethyl trisulfide, the β -cyclocitral concentration was greatly reduced to only 1% of the original concentration. Although β -cyclocitral was formed during the freezing-thawing process for cell treatment, it is biodegradable under hypoxic conditions, when the anaerobic microorganisms were available. Therefore, lower concentrations were observed for Cases C and D.

2.5 Allelopathic effects of odor compounds on algae cells

Allelopathic effects arise when cells produce autologous toxic substances which may inhibit or even damage the growth of other cells (Wang and Jiang, 2002). Many inhibitory substances secreted from *Microcystis* have been found, including alkaloids, fatty acids, peptides, alcohols, and terpenoids (Xia et al., 2008). The odor substances related to the *Microcystis* metabolism are mostly terpenoid-derived compounds, such as 2-MIB, β -cyclocitral, β -ionone, and heptanal (Harada et al., 2009). However, some odor compounds are non-terpenoid-derived ones, such as dimethyl sulfide and dimethyl trisulfide.

2.5.1 Effects on the color of *M. aeruginosa* solution

To investigate the effect of T&O compounds on the cell lysis of *Microcystis*, 1 g/L odorant chemicals were added in *Microcystis*-laden solution. Based on β -cyclocitral concentration of 5 $\mu\text{mol/L}$ (Watson et al., 2000) and 740 $\mu\text{g/L}$ in this study in natural wind-driven dense surface accumulation of *Microcystis* bloom, high concentration of T&O compounds were applied for more obvious phenomenon. As shown in **Fig. 5**, the sample without any chemicals being added remained green throughout the experiment

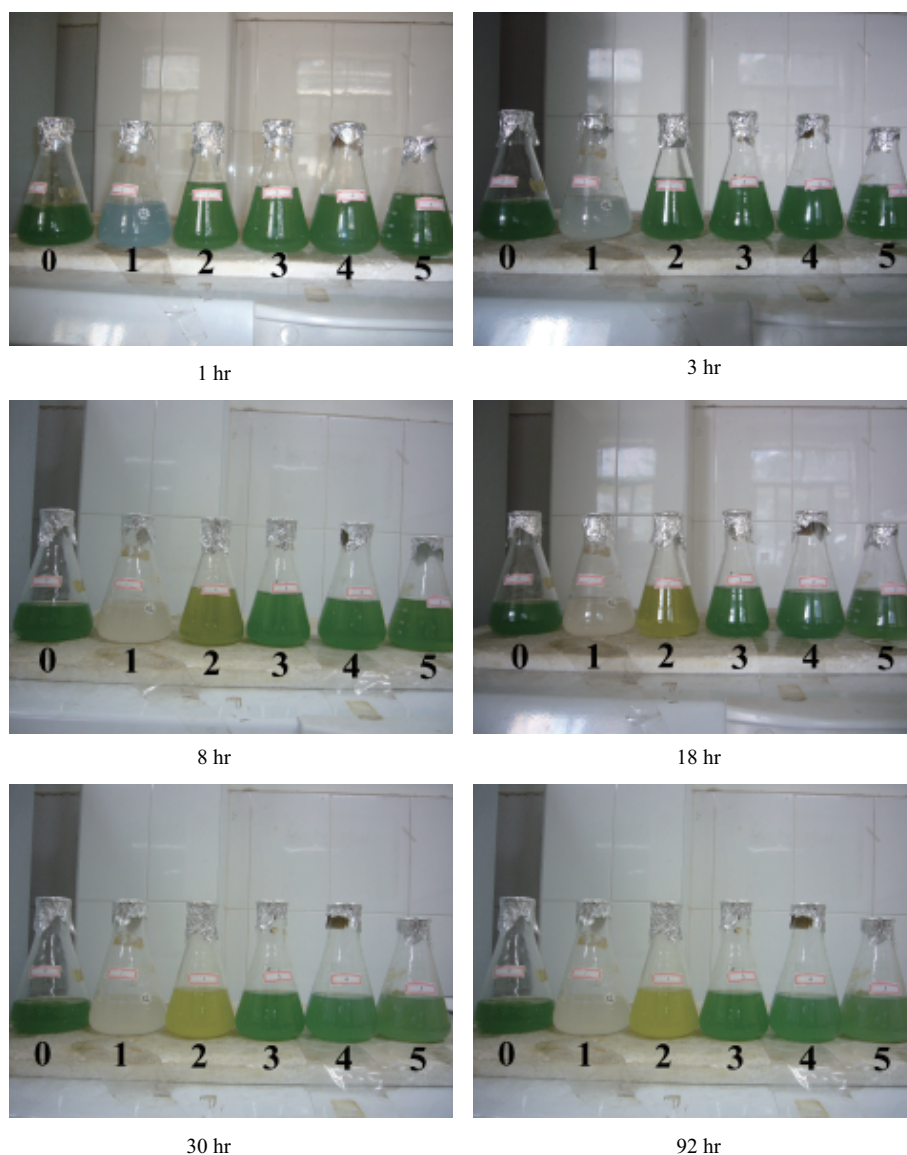


Fig. 5 Color changes in *M. aeruginosa* after spiking with different odorants. (#0) blank, (#1) β -cyclocitral, (#2) β -ionone, (#3) dimethyl sulfide, (#4) dimethyl trisulfide, (#5) heptanal. Odorant concentrations: 1 g/L; cell number: $4.7 \times 10^9 \pm 6.1 \times 10^8$ cells/L.

(92 hrs), as chlorophyll *a* is an important pigment in *Microcystis* cells. However, a few samples turned to blue at different times, including Bottle #1 at 1 hr, and Bottles #4 and #5 at 92 hr. Harada et al. (2009) reported that β -cyclocitral may lyse cyanobacterial cells and cause a change in color from green to blue, which is similar to our Bottle #1 (with the addition of β -cyclocitral). However, the addition of two other odorants, dimethyl trisulfide (Bottle #4) and heptanal (Bottle #5) may also result in the same color change, although at a much slower reaction rate. The blue color pigment, phycocyanin, is more resistant to decomposition in the solution compared with chlorophyll and β -carotene (Harada et al., 2009), and thus the solution was blue. After the blue pigment decomposed, the color disappeared and the solution became clear, as shown in Bottle #1 at 3 hr of reaction time.

For Bottle #2 (with the addition of β -ionone), the color

changed from green to yellow-green after 8 hr, and the yellow-green color remained unchanged until the end of the experiments. This suggests that β -ionone may cause some reduction of chlorophyll-*a* in the system, but at a slow rate. For Bottle #3, the color was almost unchanged throughout the experiment, similar to Bottle #1, suggesting that the presence of dimethyl sulfide did not affect the cells much within the time scale of our experiments. Ozaki et al. (2008) reported that β -cyclocitral is the only volatile substance to induce the color changes in algae solution. In addition to β -cyclocitral, other odorants, β -ionone, heptanal, and dimethyl trisulfide, may also cause changes in the color of *M. aeruginosa* solution.

2.5.2 Effects on cell activity

In the experiments examining cell activity, absorbance OD_{680} is used to represent the number of cyanobacterial

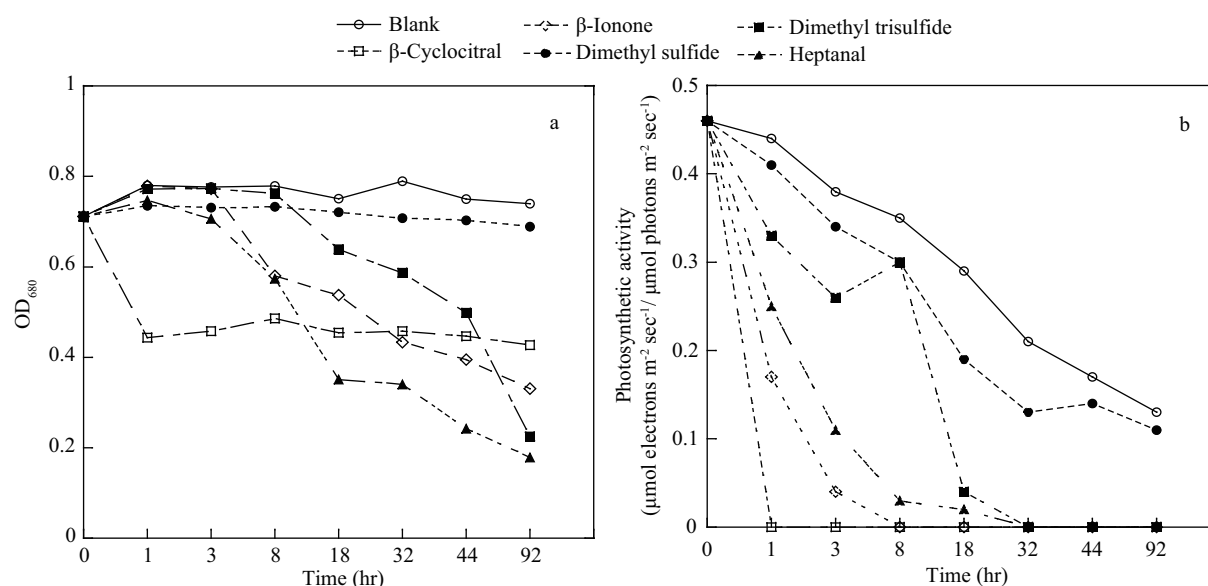


Fig. 6 Changes of cell properties after treating with different odorants. (a) absorbance at 680 nm, (b) photosynthetic efficiency.

cells, and the light efficiency is measured by PAM to assess photosynthetic activity. **Figure 6a** shows the OD₆₈₀ of the cyanobacteria-laden solution after addition of 1 g/L of different odorants. One hour after adding β -cyclocitral, the cell number (OD₆₈₀) quickly decreased by 55%, and then kept constant. At the same time, **Fig. 6b** indicates that photosynthetic activity decreased from 0.46 to 0 with the addition of β -cyclocitral, suggesting that the cells lost their photosynthetic activity. Ozaki et al. (2009) found that 1 hr after adding 1 g/L of β -cyclocitral, the surface of the algae cells began to wrinkle and become uneven, based the photos taken with scanning electron microscope. This is similar to our observation that β -cyclocitral may serve as an algacide for *Microcystis*. When adding other odorants, photosynthetic activity was also found to decrease for all cases, although to a different extent for different chemicals. Even for the blank case, photosynthetic activity also decreased slowly. However, β -cyclocitral had the greatest effects on cell activity, and thus is likely to be an important chemical for the regulation of cyanobacteria in fresh water ecosystems.

Different chemicals may have different impacts on cells, including cell morphology and cell structure. Several terpenoids, such as β -cyclocitral, are able to cause cyanobacterial cells to shrink and then wrinkle through the dissolution of cell walls or membranes (Ozaki et al., 2009). For dimethyl trisulfide, a non-terpenoids, the effects on the cells are mainly caused by acid pressure (Huang et al., 2002). In addition, a high concentration of chemicals in the solution may disrupt the permeability barrier of the cell membrane structure (Lambert et al., 2001), resulting in the expansion of cell volume and ultimately the rupturing of cells.

3 Conclusions

The concentrations of β -cyclocitral and β -carotene were found to be related to the growth phases of *Microcystis*. The correlation between β -cyclocitral and β -carotene was strong, with a correlation coefficient of $R^2 = 0.96$. β -cyclocitral is formed from the cleavage reaction of β -carotene with a production ratio of 0.16. Healthy cells were found to produce more β -cyclocitral, which may be attributed to the presence of more β -carotene in the cells. Under GC/MS analysis, more β -cyclocitral (41–865 fg/cell) was detected for live *Microcystis* cells. However, for dead cells, large amounts of sulfide compounds, especially dimethyl trisulfide, were detected at 3.48–6.37 fg/cell. β -Cyclocitral, β -ionone, heptanal and dimethyl trisulfide have inhibitory and damaging effects on *Microcystis* cells to varying extents. Among these chemicals, β -cyclocitral may quickly rupture cells, making the cyanobacteria-laden water change from green to blue.

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