Release behavior of odor contaminants derived from *Microcystis aeruginosa* in rivers and a non-strict anaerobic aqueous system

Xiaoyan Ma, Jiao Feng, Yali Song, Mengting Ni, Andrea M. Dietrich, Chen Chen, Qingsong Li and Naiyun Gao

ABSTRACT

Data were collected and reviewed to assess the odorous contaminant status of drinking water sources for Hangzhou City, China. β-Cyclocitral, β-ionone, dimethyl trisulfide, 2-methylisoborneol, and geosmin were targeted odorants. Results indicate that β-cyclocitral was the main contaminant in source waters as it was most frequently detected and occurred at higher concentrations compared to other odorants. Cyanophyta, including *Oscillatoria*, *Microcystis*, and *Anabaena*, were detected in river source waters. The origin of β-cyclocitral was also investigated in the laboratory by simulated non-strict anaerobic experiments using a prevalent species, *Microcystis aeruginosa*. Under non-strict anaerobic conditions, *M. aeruginosa* released primarily β-cyclocitral and β-ionone. Correlation of β-cyclocitral and cyanobacteria counts in the laboratory provide an explanation for high β-cyclocitral concentrations in source waters. For a *M. aeruginosa* cell concentration of 10^6 cells/L, average release and potential β-cyclocitral concentration were 49 and 44 ng/L. For 10^7 cells/L, the values increased to 725 ng/L and 545 ng/L, respectively. Environmental conditions, including temperature, pH, and illumination, exhibited substantial impact on cyanobacterial production of β-cyclocitral in aqueous systems.

Key words | β-cyclocitral, algae, cyanobacteria, *Microcystis aeruginosa*, odor metabolite, water

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INTRODUCTION

In recent years, the worldwide occurrence of algal and cyanobacterial blooms has had deleterious effects on aqueous ecosystems and also posed risks to human health from release of toxic and/or taste-and-odor compounds into source water (Li & Wan 2007). The 2007 urban water supply crises in the Chinese cities of Wuxi and Qinhuangdao were caused by odorous contaminants, including dimethyltrisulfide (DMTS), probably derived from algae and cyanobacteria; these events raised residents' fears and lowered their confidence in the water supply services (Yu *et al.* 2007; Zhang *et al.* 2010; Dietrich & Burlingame 2015). Similarly, in Zhejiang province, located on the southeast coast of China, the water quality is rapidly deteriorating due to urban development. Pollution events involving odors occurred several times in the Qiantang River which is the main water supply source for the 2.5 million residents of Hangzhou City. In July 2004, an algal bloom caused disgusting odors at both the intake and outlet of its Jiuxi Waterworks. The occurrence of odorous contaminants was concurrent with algae-laden periods in the source waters.

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Watson (2003) verified that more than 200 kinds of taste and odor (T&O) metabolites can originate from algae. The most prevalent odorous algal metabolites are geosmin (GSM; earthy) and 2-methylisoborneol (2-MIB; musty), which are reported to be produced by over 40 species of cyanobacteria (Jüttner & Watson 2007). These T&O chemicals are readily perceived by the human senses as their thresholds are 1–10 ng/L for GSM and 2-MIB (Suffet *et al.* 1996; Rashash *et al.* 1997; Lloyd *et al.* 1998; Ömür-Özbek & Dietrich 2005; Li *et al.* 2007; Piriou *et al.* 2009). Other odorous cyanobacterial metabolites include β-cyclocitral, β-ionone, 3-methyl-1-butanol, decanal, hexanal, and 6-methyl-5-hepten-2-one (Dietrich *et al.* 1995; Suffet *et al.* 1999; Zaitlin & Watson 2006; Watson *et al.* 2007, 2008). DMTS has a pungent odor of sulfurous-onion-garlic and results from cyanobacteria or decay cells and organic matter (Zimba & Grimm 2003; Ma *et al.* 2012).

The type of T&O compounds depends on the distribution of algae in the surface water, as well as water quality factors, such as nutrients, sunlight, temperature, dissolved oxygen (DO), and turbidity. Reports indicate that the amount of GSM and 2-MIB was correlated with synthesis of chlorophyll *a* for a culture of *Oscillatoria* (Tsuchiya & Matsumoto 1999; Zimba *et al.* 1999). At optimum temperatures GSM syntheses approaches zero order (Saadoun 2005). Li *et al.* (2012) found that 25 °C and 100 μmol photons $m^{-2}s^{-1}$ were beneficial conditions for the growth of three *Oscillatoria* species and the subsequent release of odor contaminants. When *Brevibacillus* sp. was co-cultured with *Microcystis*, the former promoted the production of β-cyclocitral and 3-methyl-1-butanol, and these odorants could cause lysis of *Microcystis* cells (Ozaki *et al.* 2008; Fujise *et al.* 2010; Chang *et al.* 2011). *Microcystis aeruginosa* can release aliphatic hydrocarbons (C_{15}–C_{21}), naphthalene, β-cyclocitral, and β-ionone in media containing iron under direct sunlight (Walsh *et al.* 1998). When cultured under anaerobic conditions, *M. aeruginosa* released GSM, 2-MIB, β-cyclocitral, β-ionone, and DMTS, with the intracellular concentrations of DMTS and β-cyclocitral being higher than their extracellular concentrations (Li *et al.* 2012).

To assist in identifying and solving emergency odor problems that occur in source water, an investigation of odorous contaminant production was conducted for the common cyanobacterial species *M. aeruginosa*. Specific objectives were: (1) to characterize nutrients, algae/cyanobacteria, and odorants in water sources for Hangzhou City's drinking water; and (2) to characterize odorants derived from laboratory cultured *M. aeruginosa* under simulated non-strict anaerobic conditions. The results are anticipated to provide information that will help to understand odor incidents originating from algae, especially cyanobacteria, and to be useful for controlling and reducing T&O pollution.

MATERIALS AND METHODS

Materials and equipment

The following reagents were used: GSM (≥98%, Sigma–Aldrich, USA), 2-MIB (≥98%, Sigma–Aldrich, USA), dimethyl trisulfide (≥95%, TCI, Japan), β-cyclocitral (≥99%, ALFA, USA), β-ionone (≥97.1%, AccuStandard Inc., USA), pure water (18 MΩ, Mili-Q ultrapure water), and distilled water (Zhejiang University of Technology Institute of Materials, China). *M. aeruginosa* (FACHB-905) was purchased from the Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan (cultured in BG11-type medium). Other reagents, such as sodium chloride and sodium carbonate, were analytically pure and purchased from Chinese chemical manufacturers. Sodium chloride was dried at 105 °C for 2 h before use.

The gas chromatograph-mass spectrometer was a 2010QPlus (Shimadzu, Japan) equipped with a 30 m RTX-5 ms column (Restek, USA). Other equipment included: a solid-phase micro-extraction (SPME) device (SUPELCO, USA), a digital microscope (KH-7700, Hirox, USA), magnetic stirrers (Hangzhou Electrical Instrument Company, China), and ultra-filters with microfiltration membranes (0.45 μm).

Experimental procedures

A field monitoring investigation was carried out by sampling at the water inlets of four waterworks which used different source waters to provide drinking water to the city of Hangzhou. Samples were taken over an entire year (2011)

to track the seasonal variations of planktonic algae and cyanobacteria and their odorous metabolites. The algal concentration was obtained by counting after stabilizing with Lugol's solution. Conventional physical and chemical indices of water quality were determined according to the Standard Methods for Water and Wastewater Monitoring and Analysis (Chinese, 4th Edition). Headspace–SPME/gas chromatography-mass spectrometry was used to detect and analyze the trace odorous contaminants in water.

Laboratory experiments used *M. aeruginosa* in logarithmic growth phase which were diluted with pure water to the designated concentrations, and then the cyanobacteria-laden suspensions were deoxygenated using anhydrous sodium sulfite to simulate anaerobic conditions. Suspensions were sealed with a membrane to maintain low oxygen conditions and put into an incubator. Samples were taken daily for the next 10 days through a sampling port that did not allow atmospheric oxygen to enter. The 25-mL samples were filtered through a 0.45-μm membrane and the filtrate was analyzed to determine the level of extracellular odorous metabolites and DMTS. The cyanobacteria intercepted by the membranes were frozen at −20 °C for at least 12 h to rupture the cells, then were thawed at room temperature and diluted with 25 mL water to dissolve the intracellular odorants.

Analysis methods

The 25 mL aqueous samples were placed into 40 mL vials with 30% (w/v) NaCl added. Head-space SPME was carried out using polydimethylsiloxane/divinylbenzene coated fiber (65 μm) to extract the targeted odorous metabolites at 60 °C by stirring for 30 min. The fiber was desorbed in a splitless-injector at 250 °C for 1 min in splitless mode. The gas chromatograph was programmed from 40 (held for 2 min) to 200 °C (at a rate of 10 °C/min) and finally to 250 °C (at a rate of 20 °C/min, hold for 2 min). High-purity helium was used as the carrier gas at a rate of 1.5 mL/min. For mass spectrometry, the temperature of the transfer line was maintained at 280 °C while the ion source temperature was kept at 230 °C. The selected ion modes chosen for analysis of 2-MIB, GSM, DMTS, β-cyclocitral, and β-ionone with characteristic ions were (m/z) 95 (107, 135), 112 (111, 125), 126 (79, 45), 137 (123, 109) and 177 (123, 43), and retention times were 11.21, 14.60, 7.47, 11.78 and 15.61 min, respectively.

RESULTS AND DISCUSSION

Algae/cyanobacteria and odorant distribution in source waters of Hangzhou City's drinking water

Surface waters from the Qiantang and Dongtiaoxi rivers are the principal sources of water for Hangzhou City, the former provides more than 80% of the total water demand. The Shanhusha Reservoir and Tiesha River are used as backup sources when the Qiantang River is intruded by saline water for a few days every year.

Algae/cyanobacteria and odorants distribution were investigated by sampling the inlets at four waterworks: #1 and #2 located on the Qiantang River, #3 on the Tiesha River, and #4 on the Dongtiaoxi River.

The source waters investigated were characterized by abundant nutrients, with monthly total phosphorus (TP) levels of 0.05–0.31 mg/L, total nitrogen (TN) levels of 1.44–5.48 mg/L, and NH_3-N levels of 0.20–2.74 mg/L, suggesting a high risk of algal bloom occurrence. It is widely accepted that TN and TP are closely associated with eutrophication, and that concentrations of 0.2 mg/L TN and 0.03 TP mg/L may lead to a bloom (Chen 2006). DO varied throughout the year. From December to June, it was almost saturated, while for July to November, it was distinctly unsaturated indicating that organic compounds or organisms decayed and consumed oxygen. DO concentrations in source waters were lowest in August and September at about 4 mg/L.

Five categories and 19 species of indigenous planktonic algae were identified, with Bacillariophyta and Chlorophyta found at the highest concentrations. Cyanophyta, including *Oscillatoria*, *Microcystis*, *Anabaena*, and others were found at relatively low levels with average concentration less than 7.8×10^4 cells/L. The presence of nitrogen, phosphorus, organics, trace elements, turbulence and hydrology will influence variation in cyanophyte species; nutrients and temperature are the leading factors. Considering the water quality and hydrology conditions suggests there is a risk of a cyanophyte bloom occurring

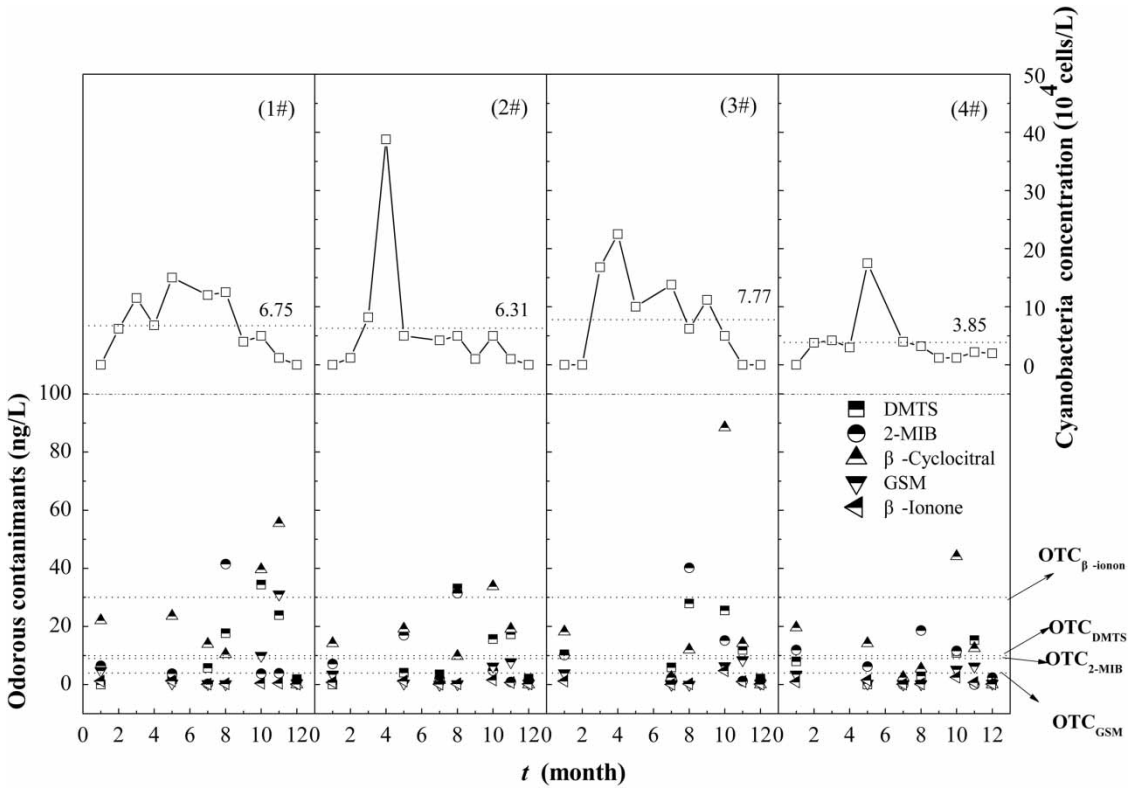

Figure 1 | Variation of cyanobacteria and odorous metabolites and DMTS at intakes to waterworks that provide drinking water for Hangzhou City. #1 and #2: Qiantang River; #3: Tiesha River; #4: Dongtiaoxi River. Dotted lines for cyanobacteria indicate the average concentration for the year. OTC of β-cyclocitral is 0.5–19 μg/L and exceeds the range shown.

in source water with potential release of odorous contaminants into the water.

Figure 1 shows the variation of cyanobacteria and odorants in source waters of Hangzhou. At intake #1 (Qiantang River), cyanobacteria maintain higher than average levels from April to August. Many odorants were detected at high levels during August to October, when the cyanobacteria start to decline. β-cyclocitral was found at the highest level (55.5 ng/L), more than any other odorous contaminant, but still under its odor threshold concentration (OTC) of 0.5–19 μg/L (Cotsaris *et al.* 1995; Young *et al.* 1999). DMTS was frequently detected and exceeded its OTC with maximum values of 34.4 ng/L, while 2-MIB and GSM were rarely detected and only once exceeded their OTCs of 1–10 ng/L. At intake #2 (Qiantang River) water was from the same source as #1, and had similar average concentrations of cyanobacteria as #1 but differed by having a sharp peak in April. Though there were lower cyanobacteria concentrations in the summer months, odorants still existed. The maximum value of DMTS (33.1 ng/L) appeared in August. The 2-MIB was also found to have its highest value (41.5 ng/L) in August, while GSM was detected at a low concentration (below its OTC), and β-ionone was hardly detected. β-cyclocitral's highest concentration (33.8 ng/L) occurred in October. At intake #3 (Qiantang and Tiesha rivers), increased algal/cyanobacterial growth started early in March, then the annual average cyanobacteria concentration was higher than water sources #1 and #2. The highest concentration of DMTS (27.9 ng/L) and 2-MIB (40.2 ng/L) occurred in August. For β-cyclocitral, its highest concentration (88.5 ng/L) occurred in October. GSM concentrations were below the OTC and β-ionone was hardly detected. Cyanobacteria concentrations and odorous contaminant levels were comparatively low in source water from intake #4 (Dongtiaoxi River). The highest concentration of 2-MIB (18.6 ng/L) was detected in August, while DMTS at 15.3 ng/L in November. The maximum value of β-cyclocitral appeared at 44.1 ng/L in October. GSM was still below the OTC and β-ionone was hardly detected.

These results demonstrate that select odorants exceeded their OTC values during 2011, usually in the summer or fall. DMTS concentrations above its OTC usually occurred between August and November, when the temperatures gradually fell, a large number of algae/cyanobacteria died, and a decline in DO to 4 mg/L occurred leading to suitable conditions for DMTS generation. A possible source of DMTS is conversion from methionine to methanethiol by the action of a demethiolase; methanethiol is the direct precursor of numerous sulfur compounds (Leduc *et al.* 2012). The 2-MIB was detected with significantly high values in August, while GSM fluctuated but was mostly below the OTC during the sampling period. β-cyclocitral was detected as a major odorant in the raw water samples from the four waterworks at higher concentrations than other odorants. There have been few reports of β-cyclocitral as a detectable odor episode since its OTC is much higher than other odor contaminants (Zhang *et al.* 2011, 2012).

To determine the relationship between cyanobacteria and the odorous contaminants, experiments focusing on algal release under unfavorable condition were carried out with *M. aeruginosa* selected as a typical precursor organism.

Characteristics of odorants derived by *M. aeruginosa* under simulated non-strict anaerobic conditions

Simulated suspensions were prepared with cyanobacteria at initial concentrations of 10^6–10^8 cells/L, which were initially deoxygenated and placed in conditions analogous to non-strict anaerobic conditions. Since the algae can produce oxygen by photosynthesis, strict anaerobic conditions cannot be maintained. The suspensions were sampled for odor detection daily. Parallel experiments were simultaneously carried out. The species and distribution of odorous compounds released by *M. aeruginosa* were detected as follows.

Volatile odor contaminants

The variability of odorous contaminants produced by *M. aeruginosa* under simulated anaerobic conditions in the laboratory is shown in Figure 2. These conditions were selected to simulate low DO situations. β-cyclocitral was the major volatile odor detected in metabolites of *M. aeruginosa*. β-ionone was detected at an extremely low level in the algal-rich water of 10^7 cells/L. Over the

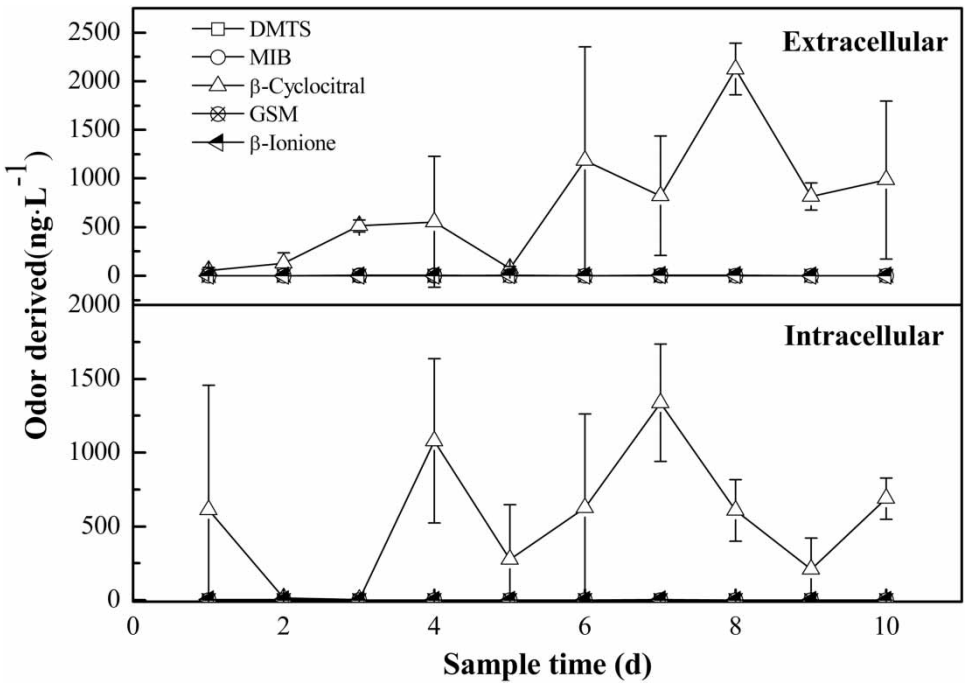

Figure 2 | Extracellular and intracellular odor contaminants with *M. aeruginosa* cells at a density of 10^7 cells/L.

experimental period, β-cyclocitral varied within the range of 55–2,124 ng/L and β-ionone was found to be less than 4 ng/L or below the detection limit. Some reports have shown that β-cyclocitral was not an original intracellular component of *M. aeruginosa* or its direct metabolite, but rather the result of oxidative degradation of β-carotene in the presence of the enzyme carotenoid cleavage dioxygenase (CCD) after cell rupture (Jüttner & Höflacher 1985; Zimba & Grimm 2003; Höckelmann & Jüttner 2005; Daiki *et al.* 2010; Jüttner *et al.* 2010). The production of β-cyclocitral and the reaction kinetics of the oxidation of β-carotene are described by Zhang *et al.* (2011). Fujise *et al.* (2010) discovered mixed odors in a culture of *M. aeuroginosa* NIES-298 including β-cyclocitral and β-ionone. β-cyclocitral was detected at about 180 μg/L on day 7 and increased continuously until day 21, after which the concentration decreased, while the detection concentrations of β-ionone, were observed at a low level. These observations are similar to those in our study. β-cyclocitral and β-ionone derived from *M. aeruginosa* are both converted from β-carotene; however, β-ionone still has double bonds in its structure which can be broken in biological or chemical reactions and converted to β-cyclocitral. Thus, β-cyclocitral was far more prevalent than β-ionone (Benevides *et al.* 2011). In fact, β-cyclocitral can originate from any algae/cyanobacteria containing β-carotene, which explains why β-cyclocitral is prevalent in source waters.

In the experiment, when the *M. aeruginosa* concentration was 10^7 cells/L, β-cyclocitral was detected above its OTC in the aqueous system, evidently produced by cells rupturing in the non-strict anaerobic conditions. The concentration is related to the number of ruptured cells, the amount of β-carotene released when they rupture, and the efficiency of the enymatic reaction. The concentration initially increased, then decreased with time. The ruptured cells reach a maximum value on day 8 characterized by a peak value of 2,124 ng/L, then declined to less than 1,000 ng/L on days 9 and 10. Intracellular quantities, detected by the freeze-thaw method, varied in the range between 5 and 1,079 ng/L during the monitoring period. The total odorants of extracellular and intercellular β-cyclocitral should not have varied based on algal counts which were constant – when the extracellular concentration increased, the intercellular should decrease, but this did not happen. Actually, the multiplication of *M. aeruginosa* under non-strict anaerobic conditions always occurs since an *M. aeruginosa* cells can release a large quantity of nutrients for growth, and the β-carotene in cells can still be transformed to β-cyclocitral in unfavorable conditions. This intracellular amount is known as release potential.

β-Cyclocitral distribution characteristics of *M. aeruginosa*

Algae-derived odors are volatile or semi-volatile contaminants, so they not only exist in the cell and in the aqueous phase, but also to a large extent in a gas phase. Based on the volatility and Henry's Law, head space-SMPE was used as the odor detection method. Figure 3 shows the distribution characteristics of β-cyclocitral in each phase under non-strict anaerobic conditions for high *M. aeruginosa* concentration (10^8 cells/L). β-cyclocitral fluctuated in the range of 105–1,484 ng/L in the aqueous phase, 1,457–15,739 ng/L as potential in cells, and 25–1,022 ng/L in the gas phase. Gas concentrations were at similar level to those of the aqueous phase, while intracellular concentrations were much higher, indicating that the majority of *M. aeruginosa* cells remained intact with β-carotene inside the cells. In addition, if β-cyclocitral is exposed and oxidized under adverse conditions, it can be released at 10 times the rate as that in the aqueous phase. When the *M. aeruginosa* cell concentration was 10^8 cells/L, the initial β-cyclocitral in the aqueous phase was lower, with release quantities being less than 304 ng/L in the first 6 days. β-cyclocitral reached the highest value of 1,484 ng/L on day 7, followed by a slight decline. Correspondingly, the initial intracellular concentration maintained a high level showing an initial increase followed by a tendency to decrease. *M. aeruginosa* proliferation occurred, causing the sum of gas phase, aqueous phase, and intracellular quantities to vary.

Factors influencing β-cyclocitral distribution characteristics

Figure 4 shows the different distribution features of β-cyclocitral with various *M. aeruginosa* concentrations. When the cell concentration was low (10^6 cells/L), β-cyclocitral had a lower release and potential amount, the maximum release concentration was 84 ng/L on day 5, and maximum

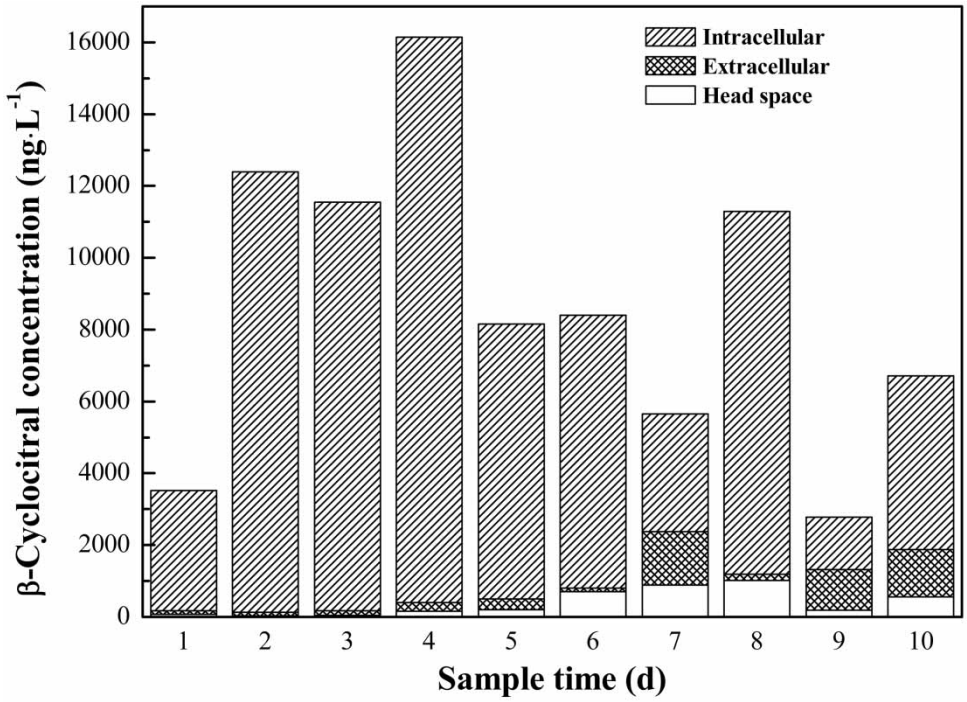

Figure 3 | β-Cyclocitral distribution over time for *M. aeruginosa* (10^8 cell per liter).

intracellular and total quantities were 220 ng/L and 262 ng/L, respectively. The latter occurred on the first day of anaerobic conditions. Extracellular concentration followed an initial increase and then decrease trend. With *M. aeruginosa* concentration at 10^7 cells/L, the highest release amount and potential were 1,183 ng/L and 1,337 ng/L, respectively. The release amount in the aqueous phase first increased and then fell, reaching a maximum on day 6 under anaerobic conditions. The intracellular potential showed a fluctuation and was at the same level as the extracellular, with a maximum occurring on day 7. When the *M. aeruginosa* concentration increased to 10^8 cells/L, the release amount in the aqueous phase was 1,484 ng/L (on day 7), the lowest figure for intracellular potential was comparable, the highest concentration was 15,739 ng/L (on day 4), far more than that in the aqueous phase.

No matter what the *M. aeruginosa* concentrations were, theoretically pure water was used to prepare the cyanobacteria suspensions and therefore cannot provide the nutrients for survival and reproduction of *M. aeruginosa.* But in the simulated non-strict anaerobic conditions, assuming no cell reproduction, released β-cyclocitral was supposed to be strictly related to the number of ruptured cells and the trend should be a gradual increase to a maximum value followed by a decrease since the cells decay gradually. However, in this study, the release and total β-cyclocitral showed no regular trend, which could be explained by considering that dead organisms were providing nutrients and conditions that allowed for survival and reproduction of *M. aeruginosa,* though the proliferation rate was low in the oligotrophic laboratory conditions (see Figure 5).

Jones & Korth (1995) reported that the average cell load of β-cyclocitral for *M. aeruginosa* is 10 fg/cell based on field sampling of *M. aeruginosa* aggregations. Zhang *et al.* (2013) reported a much higher load in the range of 41–865 fg/cell based on a culture with excess nutrients, and a range of 54–145 fg/cell for *M. aeruginosa* sampled from algae-rich Taihu Lake. In our study, the β-cyclocitral load was in the range of 34–84 fg/cell for 10^6 cells/L, 6–212 fg/cell for 10^7 cells/L and 1–15 fg/cell for 10^8 cells/L. The difference can be likely explained by both the different conditions (field vs. laboratory) and the fact that the non-strict anaerobic conditions benefit the decay of *M. aeruginosa* cells while being unfavorable to the conversion of β-carotene to β-cyclocitral.

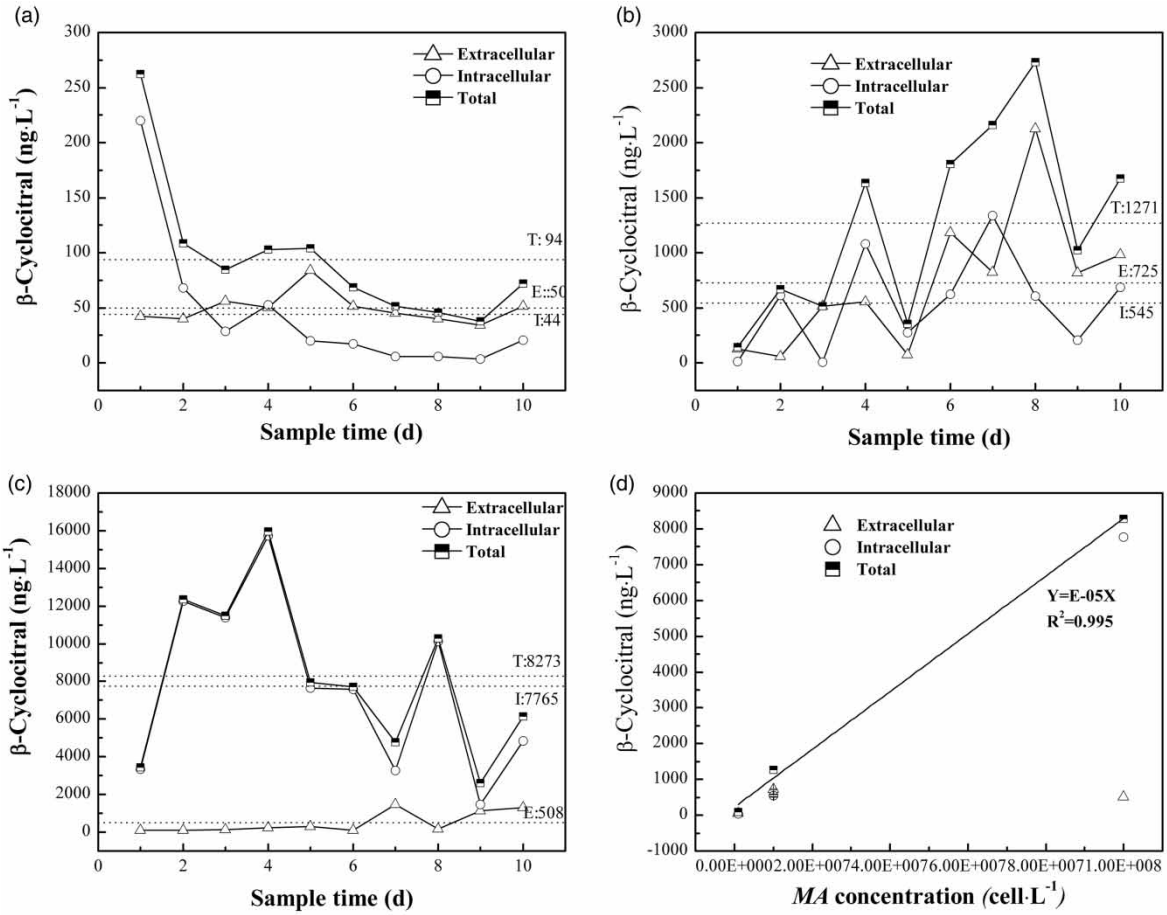

Figure 4 | β-Cyclocitral distribution at different cell concentrations (a) 10^6 cells/L; (b)10^7 cells/L; (c)10^8 cells/L).

Some reports (e.g., Wu *et al.* 2009) have shown that there is a relationship between cell concentration and pH (or DO) in the aqueous solution. The pH value of the *M. aeruginosa* culture medium increased from 7.2–8.8 to a maximum of 10.0–10.9 from the lag phase to the logarithmic growth phase, and dropped to 8.6 in the stationary phase and the decline phase. Figure 5 illustrates that under both cell concentrations, pH increased above 11 and then dropped and varied little around 10.

pH is an important characteristic of water, and one of the key factors influencing growth of *M. aeruginosa*. *M. aeruginosa* is adaptable to a wide pH range, and can adjust the pH to its preferred value (Kuang *et al.* 1994; Wang *et al.* 2004). pH influences the algal cell density, which in turn affects the release of odor contaminants. Figure 6(b) shows the production of β-cyclocitral when the pH was adjusted to 4.2 using HCl and the *M. aeruginosa* concentration was 10^7 cells/L. Adjusting the acidity initially significantly inhibited odor release by reducing cell reproduction. The amounts of odor released were below 250 ng/L during the first 8 days, while on days 9 and 10, odor release increased to 627 ng/L and 1,293 ng/L respectively. Furthermore, the average amount released was 265 ng/L, which was much lower than that from suspensions with an unadjusted pH (725 ng/L). The average intracellular amount released was 528 ng/L, far below average value of 1,270 ng/L in unadjusted suspensions.

Continuous monitoring of pH for 10 days showed that *M. aeruginosa* could adjust the pH of the aqueous phase (Figure 5). When the initial pH was 4.2 or 7.1, *M. aeruginosa* could rapidly force it to adjust to a suitable pH condition for growth (pH between 9.0 and 10.0) within a day, presumably because the *M. aeruginosa* concentration was high enough. Even once this pH was attained, the release amount and

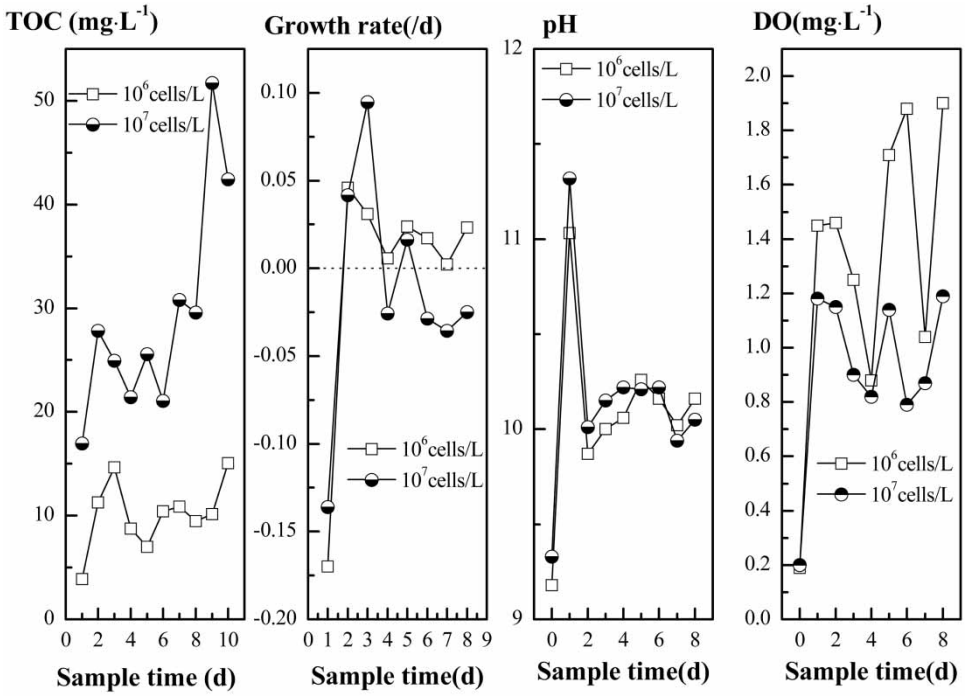

Figure 5 | Variation of pH, total organic carbon (TOC), DO and *M. aeruginosa* growth rate as a function of time.

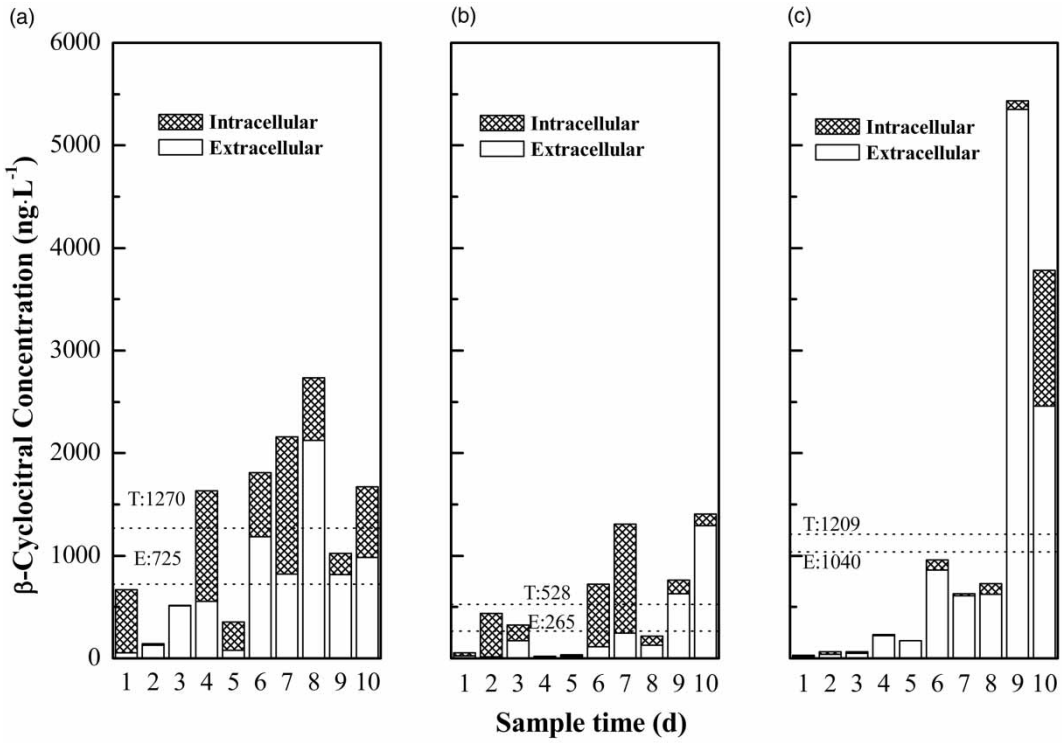

Figure 6 | Distribution of β-cyclocitral under different initial pH values of *M. aeruginosa* suspensions (initial concentration: 10^7 cells/L). (a) pH unadjusted, 12L/12D; (b) pH = 4.2, 12L/12D; (c) pH unadjusted, 24D. L, light; D, dark.

total volume of β-cyclocitral was still clearly lower for the case where the initial pH was 4.2 compared to the case where the initial pH was 7.1. The reason might be that initial HCl directly harmed *M. aeruginosa* cells and inhibited the transformation process of carotenoid under catalysis of dioxygenase.

Light was the most important factor influencing the survival of *M. aeruginosa* and β-cyclocitral release characteristics. Extracellular release of β-cyclocitral under condition of continuous darkness (days 1–5) was below 221 ng/L. Subsequently the release amount increased rapidly to 860 ng/L, and the maximum was 5,349 ng/L on day 9. The average release amount of β-cyclocitral was 1,040 ng/L, while the total amount was 1,209 ng/L in darkness. This was comparable with that found for alternating light and dark (1,270 ng/L). Hence darkness is disadvantageous for the survival of *M. aeruginosa* and leads to *M. aeruginosa* cell rupture and subsequent release of β-cyclocitral. Wang *et al.* (2013) discovered that the primary odor contaminant extracted from dead cynaobacteria (1×10^5–5×10^9 cell/L under darkness) was DMTS, and the production of DMTS (day 8) was nearly 902 ng/L in the 10^8 cells/L algae-laden suspensions. However, DMTS was not detected in our study which might be because of the source of algae species and non-strict anaerobic conditions.

CONCLUSIONS

A year-long field study demonstrated that the distribution of algae/cyanobacteria and their derived odorants in source waters for Hangzhou City were notably related to seasonal variations. Seasonal changes and temperature changes often led to organism growth and related release of odorous contaminants. Whereas the source waters are flowing rivers (the Qiantang, Tiesha, and Dongtiaoxi), the odor problems were generally low, primarily characterized by high β-cyclocitral values (but below its OTC) and DMTS and 2-MIB occasionally exceeding their OTC.

A laboratory study under non-strict anaerobic conditions investigated release of odorous contaminants from *M. aeruginosa*. Odorants released by *M. aeruginosa* were predominantly β-cyclocitral, which may explain the high β-cyclocitral in source waters for Hangzhou. The distribution of β-cyclocitral in aqueous systems and in cells varied, with the quota of β-cyclocitral being related to cell concentration and environmental factors. Environmental factors such as pH and light had a great impact on the production of β-cyclocitral in aqueous systems. Acidic conditions inhibited both released and potential concentrations, while dark conditions were observed to play an inducing role in β-cyclocitral release.

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