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Earthy odor compounds production and loss in three cyanobacterial cultures

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ABSTRACT

Geosmin and 2-methylisoborneol (MIB) related odor events caused by cyanobacteria have been a very common problem to water supply. This paper investigated the effects of temperature (18 and 25 °C) and light intensity (10 and 100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) on the production behaviors of earthy odor compounds by three odorous cyanobacteria, i.e., the geosmin-producing planktonic *Anabaena circinalis* (Ana 318), geosmin-producing benthic *Phormidium amoenum* (Pho 012) and MIB-producing benthic *Phormidium* sp. (Pho 689). At the same time, the effects of biodegradation and volatilization on the fates of the released odor compounds in water were also evaluated. The combination of high temperature (25 °C) and light intensity (100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) favored the growth of the three cyanobacteria and the production of chl-*a* and odor compounds. However, higher chl-*a* and odor yields (average odor compounds per cell) were achieved for the two benthic cyanobacteria at the temperature of 18 °C. Most of geosmin was included within the cells for Ana 318 (95–99%) and Pho 012 (85–60%), while only 20–40% MIB was bound to the cells for Pho 689. The half-life times of MIB and geosmin due to volatilization varied between 18.8 and 35.4 days, while 8 out of 10 samples exhibited a half-life time ($t_{1/2}$) for geosmin biodegradation shorter than 1 day (0.38–15.0 h), showing that biodegradation could affect the fate of geosmin significantly in aquatic environments. In comparison, biodegradation of MIB was much slower ($t_{1/2}$: 122–2166 h). Denaturing gradient gel electrophoresis (DGGE) analysis showed that *Pseudomonas*- and *Sphingomonas*-like bacteria coexisted with cyanobacteria in the cultures, and may have played an important role in geosmin/MIB biodegradation. The result of this study will be helpful for better understanding and managing the earthy odor problems caused by cyanobacteria in water supply.

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1. Introduction

Geosmin (trans-1,10-dimethyl-trans-9-decalol) and MIB (2-methylisoborneol) are two of the most frequently encountered biologically produced odor compounds in drinking water

(Watson, 2003; Watson, 2004; Zaitlin and Watson, 2006). More than forty species of cyanobacteria have been confirmed as geosmin or MIB producers, including the planktonic *Anabaena*, *Aphanizomenon*, *Planktothrix* and *Pseudanabaena*, and benthic *Phormidium*, *Oscillatoria* and *Lyngbya* (Izaguirre and Taylor,

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2004; Jüttner and Watson, 2007). Since odor problem is one of the major issues for water supply (Khiari, 2004; Suffet et al., 1996) it is important to understand the impacts of environmental factors on the production behaviors of geosmin and MIB by cyanobacteria as well as their fates in source water.

The impacts of temperature and light intensity on the growth of cyanobacteria and odor production have been extensively studied. In many cases, the production of geosmin (by *Oscillatoria brevis*) or MIB (by *Oscillatoria f. granulate*, *Pseudanabaena articulata*, *Oscillatoria*, etc.) was found to coincide with chl-*a* synthesis in responding to the changes of light and temperature conditions (Naes et al., 1985, 1988; Naes and Post, 1988; Tsuchiya and Matsumoto, 1999; Zimba et al., 1999). In some other studies, however, minimum geosmin synthesis was achieved under the optimal growth temperature (Blevins et al., 1995; Saadoun et al., 2001; Wu and Jüttner, 1988). So a comparative study using diverse cyanobacterial cultures with different ecological characteristics and odor production profiles is therefore necessary to better understand the responses of cyanobacteria to the changes of environmental conditions.

On the other hand, the fates of the odor compounds in aquatic environments are very important for water quality management in water supply industry (Taylor et al., 2006). Odor compounds produced by cyanobacteria normally were sorted as cell-bound (intracellular) and dissolved (extracellular) fractions. Once released into water, these odor compounds may disappear via biodegradation or volatilization pathways. Geosmin and MIB could be biodegraded in the sand filters of water treatment plants by bacteria including *Pseudomonas* sp. and *Sphingomonas* sp. (Elhadi et al., 2004, 2006; Ho et al., 2007; Hoefel et al., 2006; Saadoun and el-Migdadi, 1998; Saito et al., 1999). Bacteria including *Pseudomonas* sp. have also been found to coexist with *Anabaena* isolates (Aoyama et al., 1995; Lupton and Marshall, 1981). So biodegradation maybe an important process in affecting the fates of the odor compounds in water. Furthermore, volatilization is another possible process affecting the fates of these odor compounds. Until now, however, the environmental behaviors of geosmin and MIB have not yet been studied systematically, making it difficult to assess their fates in water environment.

In this paper, we investigated the effects of temperature (18 and 25 °C) and light intensity (10 and 100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) on cyanobacterial growth and odor production using three cyanobacterial cultures including geosmin-producing planktonic *Anabaena circinalis* (Ana 318) and benthic *Phormidium amoenum* (Pho 012), and MIB-producing benthic *Phormidium* sp. (Pho 689). The losses of extracellular odor compounds due to biodegradation and volatilization were also evaluated for better understanding their fates in water. The potential geosmin/MIB degrading bacteria coexisting in the cultures were revealed using PCR-DGGE followed by 16s rDNA sequencing.

2. Materials and methods

2.1. Cyanobacterial cultures

Three cyanobacteria were obtained from Australian Water Quality Centre algal culture collection, including geosmin-producing planktonic *A. circinalis* (Ana 318) isolated from Pejar

Dam, New South Wales, Australia (Bowmer et al., 1992); geosmin-producing benthic *P. amoenum* (Pho 012) isolated from Happy Valley Reservoir, South Australia; and MIB-producing *Phormidium* sp. (Pho 689, also produce a few geosmin) isolated from Lake Mathews, California, USA (Izaguirre, 1992; Zimmerman et al., 1995). The same growth medium as the AWQC uses for the preservation of each strain was employed in this study; both Ana 318 and Pho 012 were cultured in ASM medium (Gorham et al., 1964) while Pho 689 was cultured in WC medium (Robert and Guillard, 1972). Cultured Ana 318, which was dominated by double-cell segments together with a few multi-cell filaments (4–30 cells), exhibited an average cell diameter of $5.2 \pm 0.3 \mu\text{m}$ and average heterocyst cell diameter of $12 \pm 0.5 \mu\text{m}$ (photo S1 in Supporting Information). The filamentous Pho 012 has an average cell size of $3.0 \pm 0.2 \mu\text{m} \times 2.5 \pm 0.2 \mu\text{m}$ and a filament length up to several millimeters, forming compact green mats and settling at flask bottom, sometimes attaching to the flask surface (photo S2). The filamentous Pho 689 has an average cell size of $6.7 \pm 0.7 \mu\text{m} \times 3.4 \pm 0.3 \mu\text{m}$ and a filament length up to several centimeters, forming relatively loose brown mats (Photo S3). Cultures were grown in sterile 250 ml plastic tissue culture flasks (square, IWAKI® 3123-075) capped with sterile media (pore size, 0.22 μm) and incubated on orbital shakers (Ratek® OM7) within a Contherm® Digital series 1800CP incubator where both temperature and light intensity (cool white 36 W fluoro tubes) were controlled. Cultures were grown at the combination of two temperatures (25 or 18 °C) and two light intensities (100 or 10 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) using a 12 h light/12 h dark cycle. The culture series were named after the experimental conditions, for example, 25HL referring to the culture at 25 °C and high light intensity (100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). The initial cell number of test cultures was approximately 2000 cells/ml. Cultures were pre-conditioned at respective light intensities and temperatures for two weeks prior to inoculation. The orbital shakers were operated at 100 rpm with flask positions changed daily to minimize variability in light intensity. Three flasks were destructively sampled at each sampling time over a 28 day period and analyzed for cell number, chlorophyll-*a* and total and dissolved odor compounds, all assays were conducted in triplicate.

2.2. Cyanobacterial cell counting

Cell counting was performed using a Nikon® 50i microscope and Sedgewick Rafter Counting Chamber (S52, 1 ml, West Chester, USA). Lugol's solution was added to the samples allowing them to be stored and counted on a later date. Samples of Ana 318 could be counted directly since cell boundary was very clear under 40 $\times$ magnification. Samples of Pho 012 and Pho 689 were dispersed firstly using a 25 ml homogenization tube (HALU®) for 20 strokes with the pestle (about 1 min) and then using Branson Sonifier 250 with tapered microtip ($\frac{1}{8}$ ") for 10 s at 300 W. The number of cells for each of the 30 randomly selected trichomes was counted and the mean number of cells per trichome was determined. The number of cells per trichome was multiplied by the number of trichomes counted (at least 100 in total) and then converted to cell number counts. The counting error was estimated to be $\pm 20\%$ (Hötzel and Croome, 1999).

2.3. Chlorophyll-a analysis

Intracellular chlorophyll-a (chl-a) was determined with a spectrophotometric method. Following the collection of cyanobacterial cells onto a glass fiber filter (GF/C, Whatman), chl-a was extracted using 95% cold acetone and the optical absorbance of the extract was then determined at 665 nm (corrected for turbidity by subtracting the optical density measured at 750 nm) using a Cary 1 UV–visible Spectrophotometer (Varian®). The chl-a concentration was calculated using equations derived by [Wintermans and de Mots \(1965\)](#).

2.4. Odor compounds analysis

The total concentrations of MIB and geosmin were acquired by analyzing the unfiltered samples, while the dissolved ones were acquired by analyzing the filtrated samples (GF/C, Whatman). Intracellular odor compound concentrations were the difference between the total and dissolved ones. Samples were firstly pre-concentrated by solid phase micro-extraction (SPME) using a polydimethylsiloxane (PDMS) thin mat (100 µm) fiber. The adsorbed compounds were then thermally desorbed from the SPME fiber directly into the injection port of the gas chromatograph/mass spectrometer (GC/MS) and quantitatively analyzed using selected ion monitoring (SIM) mode. This analysis was carried out by an NATA (National Association of Testing Authorities, Australia) accredited laboratory. All the samples were stored in amber glass bottles at 4 °C with 40 mg/L HgCl₂ added to inhibit biodegradation.

2.5. DNA extraction and PCR–DGGE

Cyanobacterial cells of day 10 and day 14 were collected by centrifuge (8000 g, 10 min). DNA (deoxyribonucleic acid) was extracted according to xanthogenate protocol ([Tillett and Neilan, 2000](#)). PCR-DGGE (Polymerase Chain Reaction-Denatured Gradient Gel Electrophoresis) was performed using the method described in a previous paper ([Li et al., 2008](#)). The V3 region of 16S rRNA (ribosomal ribonucleic acid) gene was amplified using touchdown PCR methods as described previously by [Muyzer et al. \(1993\)](#). DGGE was performed on a D-Code apparatus (Bio-Rad, Hercules, CA) under the same conditions as described ([Muyzer et al., 1993](#)), with 30 µl of PCR products loaded. Gels were stained with ethidium bromide and visualized using UV trans-illumination (Gel Doc 2000, Bio-Rad Laboratories; Milan, Italy). The representative DGGE bands were amplified again and purified. Sequencing was done by Beijing Aoke Biotechnology Company. The sequences of DGGE bands were compared with the reference using the BLAST within GenBank database. Phylogenetic trees were constructed using MEGA version 4 by an unweighted-pair group method using average linkages (UPMGA) algorithm and the Jukes–Cantor distance estimation method with bootstrap analyses for 1000 replicates.

2.6. Volatilization test

Two hundred ml MIB and geosmin solutions were prepared by spiking MIB/geosmin standard solutions (100 µg/ml in methanol, Supelco) into sterile ASM media (40 mg/L HgCl₂ added) to

give a final MIB/geosmin concentration of approximately 1000 ng/L. Then placed them into 250 ml plastic tissue culture flasks (square, IWAKI 3123-075) capped with 0.22 µm filters and then incubated in Contherm® Digital series 1800CP incubator under dark at two temperatures (18 and 25 °C) and 100 rpm. Flasks were destructively sampled in duplicate over a 15 day period and transferred to amber glass bottles and stored at 4 °C in the dark to prevent any further loss between time of sampling and analysis. Experiments with an initial MIB/geosmin concentration of approximately 100 ng/L were also carried out at 25 °C.

2.7. Biodegradation test

When the culture experiments were finished on day 28, all the remaining culture solutions were collected and filtered through 1.2 µm GF/C glass fiber membranes to remove all cyanobacterial cells but retain bacteria partially in the filtrate. Approximately 1000 ng/L of MIB/geosmin standard solution was spiked into 2 L filtrate regardless how much odor compounds existed in the filtrates, and the spiked filtrate was transferred to 100 ml amber glass bottles immediately. Bottles were capped loosely to ensure adequate oxygen for bacteria and placed in the incubator at corresponding temperatures (25 and 18 °C) without shaking in the dark. Cultures were destructively sampled over a period of 48 h with 40 mg/L HgCl₂ added and stored at 4 °C in the dark before analysis.

2.8. Statistical analysis

The paired-samples T test was applied to compare the differences in cell growth, chl-a production and odor production between the high and low light/temperature conditions using SPSS® 20 software. Considering the non-normal distribution of chl-a and odor production data the bivariate correlation (Spearman) between chl-a and odor production in the three cyanobacteria cultures were analyzed using SPSS® too. First-order rate reactions were fitted to volatilization and biodegradation data using Origin® 8.

3. Results and discussion

3.1. Cyanobacterial growth and observed production of odor compounds

[Fig. 1](#) shows the changes in cell number (density), intracellular chl-a concentrations and chl-a yields for the three cyanobacteria under different light and temperature combinations. The highest cell densities for the three cyanobacteria were achieved under the high temperature and light intensity combination (25HL) on day 28, which was in accordance with previous observations regarding cyanobacterial growth ([Oliver and Ganf, 2002](#)). However, the two benthic cyanobacteria exhibited higher chl-a yields at the low temperature and light intensity combination, while the planktonic *Ana 318* showed similar chl-a yields under different conditions.

Observed production of geosmin/MIB by the three cultures is presented in [Fig. 2](#). Similar with the cell density results, the highest odor concentrations were also observed at 25HL:

Fig. 1 – Changes in cell number, intracellular chl-a and chl-a yields for three cyanobacterial cultures.

200 µg/L MIB, 49 µg/L and 130 µg/L geosmin for Pho 689, Pho 012 and Ana 318, respectively. Most of geosmin was intracellular for Ana 318 (95–99%), while most of MIB (60–80%) existed in the dissolved form for Pho 689, Pho 012 also kept most of geosmin within cells (85–60%), but the intracellular geosmin percentage decreased gradually with culture time. Our previous study also showed that 87–95% geosmin existed within cells in an *Anabaena spiroides* outbreak event (Li et al., 2010). So some pre-oxidation methods, which could lead to the release of intracellular odor compounds (Lin et al., 2009), may not be applicable for treating source water containing geosmin-producing cyanobacteria. In general, the MIB yield of Pho 689 was approximately 5–10 times the geosmin yields of the other two cyanobacteria. Similar with the chl-a yield results, the two *Phormidium* exhibited lower odor yields at 25 °C.

The paired-samples T test results for differences in cell growth and odor production between the high and low light/temperature conditions are presented in Table S1. The high temperature and light intensity favored the growth (cell number) of the three cyanobacteria and the production of odor compounds. The 2-tailed significances were less than 0.05 except for the temperature effects to Ana 318, suggesting that the impacts of temperature on cell growth and odor yields of Ana 318 were not as great as those of the two benthic cyanobacteria. In comparison with the benthic cyanobacteria,

Anabaena can adjust the position in water by buoyancy (Brookes et al., 1999), which allows them to choose suitable temperature and light conditions. While high light intensity was favorable for the productions of chl-a and odor compounds by Ana 318 and Pho 012 (2-tailed significance, 0.000–0.002); temperature had not significant effect on chl-a production for three cyanobacteria (2-tailed significance, 0.183–0.919). However, higher chl-a and odor yields were achieved for the two benthic cyanobacteria at the low temperature condition (Figs. 1 and 2). One similar study indicated that low temperature or light could stimulate geosmin production and favor the accumulation of geosmin in benthic *Lyngbya kuetzingii* cells (Zhang et al., 2008). Correlation coefficients (Spearman) between chl-a and odor productions for three cyanobacteria cultures were over 0.9 (0.905–0.984) with a 2-tailed significance of 0.000 (Table S2). It is known that both geosmin and MIB are produced via the isoprenoid synthesis pathway, which is also linked to chl-a formation (Ershov et al., 2000; Giglio et al., 2008, 2011; Juttner and Watson, 2007; Richard et al., 1993). This maybe the reason why the odor and chl-a yields exhibited similar responses to temperature.

3.2. Volatilization and biodegradation

The volatilization process was fitted using a pseudo first-order model, and the results are shown in Table 1. The half-life

Fig. 2 – Changes in total odor concentration, intracellular odor percentage and total odor yields.

times for MIB and geosmin due to volatilization varied between 18.8 days and 35.4 days, with temperature exhibiting larger impacts on MIB than geosmin. So volatilization may not be an important process for the loss of the odor compounds in water bodies under normal conditions. This result was in accordance with a previous study which has found that the Henry's law constants of geosmin and MIB are relatively small (0.0028 ± 0.0003 and 0.0028 ± 0.0002 at 20°C , 0.0054 ± 0.0005 and 0.0042 ± 0.0002 at 25°C , respectively) (Omur-Ozbek and

Dietrich, 2005). It should be noted that the hydrodynamic and weather conditions may also affect the volatilization process of odor compounds in actual water bodies, which was not be estimated in this study.

The geosmin/MIB biodegradation process was also fitted using a pseudo first-order model and the results are shown in Table 2 and Fig. S1. Among the 10 filtrate samples, eight exhibited a $t_{1/2}$ value shorter than 1 day with one sample having the $t_{1/2}$ as short as 0.38 h, suggesting that

Table 1 – Results of the first-order kinetics fitting for volatilization.

	Temperature ($^\circ\text{C}$)	A		K_v		R^2	$t_{1/2}$
		Value	SD	Value	SD		
MIB	25	1330	21.2	−0.0368	0.0024	0.963	18.8
	25	136.4	2.3	−0.0356	0.0025	0.957	19.5
	18	1034	9.6	−0.0196	0.0018	0.907	35.4
Geosmin	25	1165	17.0	−0.0330	0.0022	0.962	21.0
	25	118.9	1.4	−0.0283	0.0017	0.969	24.5
	18	878.3	8.2	−0.0341	0.0028	0.925	20.3

Parameters determined from fitting first-order kinetic equation $C = A \times \exp(K_v t)$, where C: concentration of geosmin/MIB (ng/L) after time t, A: initial odor concentration in water, K_v : volatilization rate, and t: time (day). SD, standard deviation; R^2 , fit of equation to data; $t_{1/2}$, half-life of odor compounds in water.

Table 2 – Results of the first-order kinetics fitting for geosmin biodegradation.

Cyanobacteria		Serial	A		K _b		R ²	t _{1/2}
			Value	SD	Value	SD		
Geosmin	Ana 318	25HL	978.5	24.25	−0.2152	0.0110	0.983	3.22
		25LL	1066	29.74	−0.1393	0.0089	0.977	4.98
		18HL	831.0	2.34	−1.838	0.0555	0.999	0.38
		18LL	1057	24.45	−0.6276	0.0356	0.991	1.10
	Pho 012	25HL	22,759	191.3	−0.0035	0.0004	0.761	200
		25LL	1149	32.06	−0.0182	0.0022	0.841	38.1
		18HL	1948	109.0	−0.243	0.0034	0.888	2.85
		18LL	697.1	18.14	−0.0906	0.0069	0.981	7.65
	Pho 689	25HL	1324	16.06	−0.0463	0.0018	0.989	15.0
		25LL	1158	23.14	−0.0495	0.0031	0.971	14.0
MIB	Ana 318	25HL	1254	12.91	−0.0029	0.0005	0.589	239
		25LL	1581	9.03	−0.0027	0.0003	0.809	257
		18HL	930.1	13.89	−0.0031	0.0008	0.440	224
		18LL	876.8	4.37	−0.0017	0.0002	0.688	407
	Pho 012	25HL	1383	9.27	−0.0024	0.0003	0.720	289
		25LL	1259	8.65	−0.0021	0.0003	0.643	330
		18HL	1145	2.98	−0.00043	0.00003	0.868	1610
		18LL	1865	4.84	−0.00032	0.00004	0.788	2166
	Pho 689	25HL	140249	1394	−0.0057	0.0006	0.849	122
		25LL	122203	894	−0.0043	0.0004	0.863	161
		18HL	91,086	445	−0.00089	0.0007	0.884	778
		18LL	113380	872	−0.0011	0.0001	0.822	630

Parameters determined from fitting first-order kinetic equation $C = A \times \exp(K_b t)$, where C: concentration of geosmin (ng/L) after time t, A: initial geosmin concentration in water, K_b: biodegradation rate, and t: time (h). SD, standard deviation; R², fit of equation to data and t_{1/2}, half-life of geosmin in water.

biodegradation should be a very important process responsible for the loss of geosmin in water bodies. Two filtrates of the Pho 012 cultures exhibited relatively low biodegradation rates (t_{1/2} values, 38.1 and 200 h). It maybe possible that the tight mats formed by the filamentous cyanobacteria have intercepted the geosmin-degrading bacteria during membrane filtration, resulting in a very low bacterial concentration in the filtrates. However, the t_{1/2} values of geosmin in the two filtrates were still much smaller than those acquired in the volatilization tests, showing that biodegradation is a more important process for geosmin.

Ho et al. (2007) recorded t_{1/2} values of similar magnitude for geosmin degradation (2.4–13.9 h) in batch bioreactors inoculated with bacteria taken from a sand filter used for treating reservoir water with a history of geosmin production by *A. circinalis*. Hoefel et al. (2009) has succeeded in isolating a geosmin-degrading bacterium, *Sphingopyxis* sp., from the same sand filter, which exhibited a t_{1/2} value as low as 0.014–0.042 h for geosmin degradation. Some other studies (Hayes and Burch, 1989; Izaguirre and Taylor, 2007) have shown that significant decrease of geosmin occurs following the disappearance of the geosmin-producing cyanobacterial blooms. So it is possible that the rapid disappearance of geosmin in water bodies may have mainly been caused by biodegradation. On the other hand, the low dissolved/intracellular geosmin ratio may also be a result of rapid biodegradation of dissolved geosmin.

For MIB, however, biodegradation was much slower in all of the filtrates with a t_{1/2} value varying from 122 to 2166 h (approximately 5–90 days). The highest biodegradation rate

was recorded in the 25HL Pho 689 filtrate, while the lowest one was in the 18LL Pho 012 filtrate. MIB appeared to be much more difficult to degrade than geosmin (Elhadi et al., 2004; Ho et al., 2007). Izaguirre et al. (1988) found that it took several months for bacteria to degrade MIB of 10–20 mg/L. At the same time, MIB has also been found to be more difficult for oxidation by ozone (Ho et al., 2004, 2002) and adsorption by activated carbon (Newcombe and Cook, 2002). It was speculated that more planar molecular structure and lower solubility made it easier to remove geosmin by oxidation, adsorption and biodegradation (Ho et al., 2007). It has been proved that high concentrations of geosmin are normally observed during *Anabaena* blooms only (Hayes and Burch, 1989; Izaguirre and Taylor, 2007; Li et al., 2010), while the MIB problem could last over the whole year in warm areas (Tung et al., 2008). So in comparison with geosmin, MIB is in general less susceptible to biodegradation in aquatic environment.

3.3. PCR–DGGE analysis

The PCR–DGGE profiles for the extracts from the three cyanobacterial cultures are presented in Fig. 3. The sequence of each representative band was analyzed (Table S3), and the phylogenetic tree was constructed by comparing the DGGE band sequences with 16s rDNA database of GenBank (Fig. 4). Bands A and J represent the *Phormidium* and *Anabaena* partial 16s rDNA genes, respectively. Fig. 4 shows that various bacteria coexisted with cyanobacteria in all of the three cultures. Band D represents bacterium related with *Pseudomonas* sp. which was only observed in the *Phormidium* cultures. Sequences

Fig. 3 – DGGE profiles for three cyanobacterial cultures at different growth periods. Letters A–L represent the 16s rDNA bands of interest.

related with *Hydrogenophaga* sp. and *Sphingomonas* sp. were found in both the *Phormidium* and *Anabaena* cultures (bands C, B and K). Bacteria including *Pseudomonas* sp. have also been observed in other uni-algal laboratory cultures of cyanobacteria (Lupton and Marshall, 1981). Worm and Sondergaard (1998) observed a dynamic heterotrophic bacteria community attaching to *Microcystis* in a eutrophic lake. *Pseudomonas* has been found to exist extensively in lake water (Izaguirre et al., 1988), biological granular activated carbon (Oikawa et al., 1995), soil (Saadoun, 2005), and water treatment sand

Fig. 4 – Phylogenetic tree of DGGE band sequences and reference sequences.

filters (Ho et al., 2007). Ho et al. (2007) found that strains related with both *Pseudomonas* sp. and *Sphingomonas* sp. in sand filters of Morgan water treatment plant, South Australia, could degrade both MIB and geosmin. So the presence of these *Pseudomonas* and *Sphingomonas*-like bacteria in the three culture systems maybe responsible for the degradation of geosmin and MIB in water.

4. Conclusions

This study investigated the effects of temperature and light intensity on the production behaviors of earthy odor compounds by three cyanobacterial cultures, and the potential impacts of volatilization and biodegradation on geosmin/MIB fates. The following conclusions were obtained:

- (1) In general, odor compounds increasing contributed by odorous cyanobacteria cell number climbing which favorite higher temperature and light intensity, while higher odor yields were acquired at 18 °C for the two *Phormidium*, which was in accordance with the chl-*a* yields.
- (2) Probably, as a result of fast biodegradation, most of geosmin was included within cells, showing that it is important to keep the intact of geosmin-producing cyanobacterial cells during water treatment. High dissolved MIB ratio maybe as a result of slow biodegradation and continuous accumulation.
- (3) Biodegradation of extracellular geosmin was much faster than MIB, and the coexisting *Pseudomonas*- and *Sphingomonas*-like bacteria maybe important geosmin-degrading bacteria. The effect of volatilization to the fates of geosmin and MIB could normally be neglected in comparison to biodegradation.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.watres.2012.06.008](https://doi.org/10.1016/j.watres.2012.06.008).

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