

1 MIB-derived odor management based upon hydraulic regulation
2 in small drinking water reservoirs: principle and application

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5 **Abstract**

The musty odorant (2-methylisoborneol, MIB) is prevalent in source water reservoirs and has become one of the major challenges for drinking water quality. This study proposes an approach to control the growth of MIB-producing cyanobacteria in a small reservoir based on hydraulic regulation, according to the results of long-term field investigations, laboratory culture experiments, model construction, and field application. Field investigations found that longer hydraulic retention time (HRT) is a factor that triggers MIB episodes. The culture study revealed that the maximum cell density, growth rate of MIB-producing *Planktothricoides raciborskii*, and MIB concentration are determined by the HRT ($R^2 = 0.94$, p -value < 0.001) and can be minimized by decreasing the HRT to less than 10 d . On this basis, an HRT regulation model was constructed and validated by field investigation, and critical HRT values were evaluated for 14 cyanobacteria genera. By decreasing the HRT to $5.4 \pm 0.8 d$, which is lower than the critical value of $7.5 \sim 15.0 d$, an MIB episode was successfully terminated in ZXD Reservoir in 2021. The results suggest that the proposed principle can provide a scientific basis for HRT regulation, which has been proved to be effective and feasible. This approach avoids negative impacts on water quality, does not require extra investment in engineering infrastructure, and in some cases may be applied readily by changing existing operational procedures. Therefore, HRT-based regulation is

a promising strategy targeting MIB control and possibly for other cyanobacterial-derived water quality problems in small reservoirs.

6 *Keywords:* 2-methylisoborneol, cyanobacterial control, HRT, drinking water, hydraulic
7 regulation, odor

8 **1. Introduction**

9 Earthy/musty odor episodes in source water reservoirs caused by 2-methylisoborneol (MIB), a
10 terpene derivative with an odor threshold concentration (OTC) as low as 10 ng L⁻¹, are often a
11 major concern for the drinking water industry (Izaguirre and Taylor, 2004; Jüttner and Watson,
12 2007; Watson, 2004). Activated carbon adsorption has been widely used for MIB removal (Li
13 et al., 2019; Zamyadi et al., 2015). However, it does not perform well when the MIB concentra-
14 tion in source water exceeds 200 ng L⁻¹ (Gillogly et al., 1999), particularly when natural organic
15 material (NOM) is abundant and competes for adsorption sites on activated carbon (Wang et al.,
16 2020). In addition, it substantially increases the operating cost for water treatment plants and
17 produces large amounts of sludge requiring disposal (Huang et al., 2020; Li et al., 2019). There-
18 fore, it is highly desirable to find options to control the growth of MIB-producing cyanobacteria
19 in source water.

20 Though MIB was first identified as the volatile secondary metabolite produced by actino-
21 mycetes, some fungi and symbiotic bacteria, filamentous cyanobacteria have been identified as
22 the major sources of MIB in drinking water reservoirs (Gerber, 1983; Jüttner and Watson, 2007;
23 Cao et al., 2023). More than 20 cyanobacterial strains of genera including *Pseudanabaena*
24 (Zhang et al., 2016; Su et al., 2021a), *Planktothrix* (Su et al., 2015), *Phormidium* (Izaguirre
25 et al., 2007), *Oscillatoria* (Van Der Ploeg et al., 1995) and *Planktothricoides* (Te et al., 2017;
26 Lu et al., 2022) have been reported to be the main MIB producers as summarized by Su et al.
27 (2021a). In comparison with the more prevalent surface bloom-forming genus *Microcystis*

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28 with small spherical cells (diameter: 3 ~ 9 μm), most of MIB-producing strains are filamentous
29 cyanobacteria (length: 1.3 ~ 12 μm , width: 0.8 ~ 15 μm) (Xu et al., 2020). Larger cellular
30 planar surface area enables them to capture more irradiance to operate under lower light
31 conditions (Su et al., 2014). In addition, they often possess more accessory photosynthetic
32 pigments (e.g. phycoerythrin) that broaden their capacity to absorb irradiance across the visible
33 spectrum through chromatic acclimation (Wiltbank and Kehoe, 2019; Su et al., 2023). These
34 characteristics allow them to grow in subsurface/deep layers where light intensity is often
35 relatively low (Halstvedt et al., 2007; Su et al., 2019) and green light and nutrients (Dokulil and
36 Teubner, 2012; Su et al., 2015) are more abundant (Wiltbank and Kehoe, 2019). Consequently,
37 they also tend to grow slower than the surface bloom-forming cyanobacteria (de Araujo Torres
38 et al., 2015; da Anunciação Gomes et al., 2015).

39 Several strategies have been developed to prevent or suppress cyanobacterial blooms, includ-
40 ing reduction of nutrient loads, chemical algaecides, physical removal, artificial mixing and bio-
41 manipulation (Everall and Lees, 1997; Greenfield et al., 2014; Fastner et al., 2015; Huisman et al.,
42 2018; Newcombe, 2012). However, the effects are not guaranteed although they have been
43 successfully in at least some lakes. Nutrients management requires long term and basin scale
44 actions (Jeppesen et al., 2005). The use of algaecides in natural water bodies and drinking water
45 reservoirs is not desirable due to potential adverse ecological impacts (Kibuye et al., 2021). Arti-
46 ficial mixing of lakes is an effective hydraulic approach to prevent blooms of buoyant cyanobacte-
47 ria but very costly (Visser et al., 1996, 2016). More specifically, the MIB-producing cyanobacteria
48 are usually not the dominant species (Su et al., 2019), leading to low control efficiencies for these
49 traditional approaches.

50 The control of subsurface/deep-living cyanobacteria in source water is not often discussed.
51 The important issues in this context are: 1) chemicals are undesirable or are restricted for appli-
52 cation in source water, and 2) most MIB-producers grow at subsurface layer with relatively low
53 abundances. In view of their unique niche characteristics, water level and turbidity regulation-
54 based strategies for cyanobacteria have been developed and verified in Miyun Reservoir and for
55 Qingcaosha (QCS) Reservoir (both in China) in our previous studies (Su et al., 2017; Jia et al.,

56 2019; Su et al., 2022). These verified applications suggest that it is feasible to minimize the
57 growth of MIB-producers in source water by reducing the underwater light intensity to below
58 their minimal requirements. However, these approaches require abundant water and capacity
59 to adjust the water level or sufficient highly turbid inflow to regulate the water turbidity, which
60 are not available for many reservoirs. In addition, the results of field monitoring in East Taihu
61 Lake and cultural experiment confirmed that submerged macrophytes can significantly inhibit
62 the growth of MIB-producing cyanobacteria through nutrient depletion, increasing water trans-
63 parency, shading and allelopathic effects, but it is more applicable to shallow lakes (Yang et al.,
64 2023).

65 Hydrodynamics has been verified as a key factor influencing cyanobacterial blooms in several
66 riverine ecosystems (Mitrovic et al., 2003; Verspagen et al., 2006; Mitrovic et al., 2011; Cha et al.,
67 2017; Romo et al., 2012). A mechanical model predicted that flushing with fresh water will sup-
68 press *Microcystis* populations when the current flushing rate is sufficiently increased (Verspa-
69 gen et al., 2006). On the contrary, rapid flow rates may prevent the formation of cyanobacte-
70 rial blooms by disturbing and diluting cyanobacterial populations and/or by increasing turbid-
71 ity, weakening thermal stratification in the water column, thereby reducing light exposure to
72 cyanobacteria (Cha et al., 2017). A discharge of 300 ML d⁻¹ (0.03 m s⁻¹) are found to be suffi-
73 cient to suppress the development of *Dolichospermum circinalis* (formerly named as *Anabaena*
74 *circinalis*), and discharge of 3000 ML d⁻¹ (0.3 m s⁻¹) can effectively remove an established bloom
75 (Mitrovic et al., 2011). These studies have demonstrated the control effect of bloom-forming
76 cyanobacteria based on large-scale flowrate increases in river system. Except a case study in
77 a shallow Mediterranean lake (Romo et al., 2012), that attributed the increase of *Microcystis*
78 *aeruginosa* biomass and microcystin concentration by 1-2 orders of magnitude to the flowrate
79 decrease during the dry season. No study has explored the effect of hydraulic regulation in
80 reservoir and lake systems, which have much lower flowrates. Besides, two aspects of hydrody-
81 namic including *in-situ* flow velocity and physical dilution may affect the growth and control of
82 cyanobacteria, the corresponding causal mechanisms, however, have not been elucidated.

83 According to our previous studies (Jia et al., 2019; Lu et al., 2022) and other publications (Ta-

84 ble '!!!!TODO KEY ERROR: tbl-refdfWAS NOT FOUND!'), the growth rates of filamentous producers
85 are relatively lower than bloom-forming cyanobacteria. Since MIB is mainly produced by fila-
86 mentous cyanobacteria, we proposed the hypothesis here: the growth of MIB-producers with
87 relatively low growth rates, can be suppressed by hydraulic regulation in reservoir systems, al-
88 though their flowrates are much lower than river systems. In this study, the effects of HRT on
89 the production of MIB were first investigated in a small reservoir in Zhuhai City, China, and veri-
90 fied in a culture experiment using an MIB-producing *Planktothricoides raciborskii* (*P. raciborskii*)
91 strain. Subsequently, a mechanical model was developed to evaluate the critical HRT values for
92 limiting the growth of filamentous cyanobacteria genera and validated in another small reser-
93 voir in Zhuhai that supplies source water to Macao. The result of this study may provide a new
94 approach and principle for controlling the MIB problems in source water, particularly for small
95 reservoirs where regulation of HRT can be achieved relatively easily.

96 **2. Materials and Methods**

97 *2.1. Study sites*

98 Two subtropical mesotrophic reservoirs, Nanping Reservoir (NP, 22°13'N, 113°29'E) and Zhux-
99 iandong Reservoir (ZXD, 22°12'N, 113°31'E), were selected and investigated in this study. Both
100 reservoirs are located in Zhuhai city, China (Fig. 1), and are charged from Xijiang River. NP Reser-
101 voir is used as the drinking water resources for Zhuhai and sometimes for Macao, while ZXD
102 Reservoir is mainly used as the drinking water resource for Macao. The mean water depths are
103 15.80 ± 12.77 m (mean \pm sd) for NP Reservoir and 6.72 ± 2.29 m for ZXD Reservoir. The dynamics
104 of water temperature followed the same seasonal pattern, with the annual mean temperature
105 of 24 °C. Weak stratification were observed with 3~5 °C temperature differences between sur-
106 face water and bottom water for both reservoirs (Fig. 2). The effective volumes of the two
107 reservoirs are 5.74×10^6 m³ (NP) and 2.61×10^6 m³ (ZXD), and the hydraulic residence times
108 (HRTs) are in the ranges of 10 ~ 55 *d* (NP) and 5 ~ 23 *d* (ZXD), respectively. NP Reservoir has

109 suffered from MIB-derived musty odor problems every spring and summer since 2017, and an
110 MIB episode was observed in ZXD Reservoir during March and April of 2021.

111 2.2. Sampling procedure

112 Since thermal stratification was weak, the samples were mainly collected from surface layer
113 (0.5 m). Long-term routine monitoring of NP Reservoir was conducted every month from 2017 to
114 2020. Water samples (1 L) were collected from the surface layer (0.5 m depth) of NP03 (near the
115 outlet), and the temperature was measured using a mercury thermometer *in-situ*. In addition,
116 two intensive investigations were performed during the two MIB episodes (July 2017 and April
117 2018) in NP Reservoir, with surface water samples (0.5 L) taken from 6 sampling sites (NP01 ~
118 NP06) every day for odorant analysis. During the MIB episode in ZXD Reservoir (March ~ April
119 2021), an intensive investigation was performed, and 0.5 L surface water samples were collected
120 every day at 6 sampling sites (ZXD01 ~ ZXD06) for quantification of phytoplankton and odorants.
121 In addition, to characterize the overall water quality of ZXD Reservoir, 0.5 × 3 L water samples for
122 the nutrients and odorants quantification were collected from three layers (surface, half depth,
123 bottom) at each site once a week in April 2021. Detailed sampling description is summarized in
124 Table '!!TODO KEY ERROR: tbl-smpdfWAS NOT FOUND!.

125 The water samples were sealed in brown narrow-mouth sampling bottles and taken to the
126 laboratory for pretreatment within four hours. The water depth (Depth) was measured using
127 an ultrasonic sounder (SM-5, Japan), and the water transparency (SD) was determined using a
128 standard Secchi disk. A multiparameter water quality probe (YSI6600, US) was used to measure
129 water temperature (Temp.), dissolved oxygen (DO), pH, turbidity (Turb.), salinity, conductivity
130 (Cond.), total dissolved solids (TDS), chlorophyll *a* (Chl *a*), phycocyanin (PC), and redox potential
131 (ORP), all parameters were calibrated in the laboratory or in the field before use according to
132 the instrument manual.

133 *2.3. Laboratory water quality analysis*

134 Water samples were kept in refrigerated (4 °C) in the dark before analysis and all water quality
135 indicators were analyzed quantified within 48 hours. Raw and filtered (0.7 µm, Whatman,
136 UK) water samples for odorants (MIB and geosmin) analysis were preserved by adding NaClO
137 (2 mg L⁻¹) to inhibit biodegradation (Fan et al., 2018). The total MIB (tMIB) and geosmin
138 (tGSM) concentration were estimated using the raw water samples, while the dissolved MIB
139 (dMIB) and geosmin (dGSM) were measured using the filtered samples. The odor compounds
140 (MIB, geosmin) were analyzed using solid phase microextraction (SPME) combined with gas
141 chromatography-mass spectrometer (GC-MS, Agilent 7890, US) (Su et al., 2015). The total
142 nutrients including total nitrogen (TN), total phosphorus (TP), ammonia (NH₄-N) and nitrate
143 (NO₃-N) were analyzed according to the national standard methods (Ministry of Environmental
144 Protection of PRC 2007, 2009, 2012, 2013).

145 *2.4. Phytoplankton quantification*

146 Raw ZXD Reservoir subsamples (100 mL) were preserved with 5% Lugol's iodine and allowed to
147 settle for 72 hours. The top 90 mL solution was then removed to achieve a 10× concentration so-
148 lution. Phytoplankton cell counting was performed using a microscope (Olympus, BX51, Japan)
149 under bright field with a counting tool (CCT V1.4, China, <https://drwater.rcees.ac.cn>). The spe-
150 cific quantification method was consistent with our previous reports (Su et al., 2015; Jia et al.,
151 2019). Chl *a* concentration of the long-term routine monitoring samples from NP Reservoir was
152 measured with raw water using AlgaeLabAnalyser (BBE, German).

153 *2.5. The growth of Planktothricoides raciborskii under different HRTs*

154 *Planktothricoides raciborskii* (*P. raciborskii*), the most abundant MIB-producing cyanobacteria
155 genus in Zhuhai Reservoirs (Fig. 10), was isolated from FH Reservoir in Zhuhai in our previous
156 study (Lu et al., 2022), and used in the culture experiment in this study. A pure *P. raciborskii* strain
157 was first cultured until the logarithmic phase in BG11 medium (30 °C, 54 µmol photon m⁻² s⁻¹),

158 then filtered using a 1.2 μm membrane (Millipore, USA), and washed three times with ultrapure
159 water for later inoculation. The experiments were performed at 6 HRT levels (2, 5, 10, 20, 40, 80
160 d) with three replicates, and named as RT02, RT05, RT10, RT020, RT40 and RT80. Filtered (0.7
161 μm , Waterman, UK) and sterilized (121°C, 30 minutes) raw water from NP Reservoir was used
162 as the culture medium (TDN: 860 $\mu\text{g L}^{-1}$, TDP: 21 $\mu\text{g L}^{-1}$). The culture experiment lasted for 18
163 d under the optimal temperature and light intensity (30 °C, 54 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$) (Lu et al.,
164 2022), which closed to the mean light intensity at 0.9 m depth in NP Reservoir during the MIB
165 episode. Since the cell densities of culture samples are relatively high, no enrichment is required
166 for optimized cell counting. In order to minimize the effect of sampling on culture system, only
167 20 mL culture samples were taken every two days for cell enumeration and odorant analysis.
168 More detail of the experiment design is provided in Fig. 3 and Table '!!TODO KEY ERROR: tbl-
169 cultureexpdesignWAS NOT FOUND!.

170 2.6. Data analysis

171 The mean actual reservoir HRT (τ , d) and specific growth rate of *P. raciborskii* from culture
172 experiments were calculated using the following equations:

$$\tau = \frac{V}{\sum Q_i} \quad (1)$$

$$\mu = \frac{\ln y_{t_2} - \ln y_{t_1}}{t_2 - t_1} \quad (2)$$

173 Where V is the volume of the actual reservoir, Q_i is the inflow. y_{t_2} and y_{t_1} are the *P. raciborskii*
174 cell densities of culture experiment at the beginning (t_1) and end (t_2) of the logarithmic growth
175 phase.

176 The growth rates of 14 typical cyanobacterial genera were determined by culture experiments
177 from published papers. In total of 1,480 records were validated and used to evaluate the statis-
178 tical distributions of their growth rates. Note that *Pseudanabaena* was identified as the main

179 MIB producer in ZXD Reservoir, where the field application was performed. The median growth
180 rate of *Pseudanabaena* based on the records from literatures (including our previous studies)
181 was used.

182 All data analysis and visualization in this investigation were based on R Language (R Core Team,
183 2021). Data statistics, statistical tests, linear modeling, and multivariate nonlinear regression
184 were completed using **base** packages (R Core Team, 2021). More specifically, two strategies
185 were performed for statistical tests, including variance analysis (ANOVA) for data meeting mul-
186 tivariate normality and variance chi-squared, and wilcoxon test for the remaining data. The
187 description of statistical test is given in the tables in the Supplementary Material. The cor-
188 relation coefficient's calculation and significance test were completed using the **Hmisc** package
189 (Harrell Jr, 2022). The graphs were drawn using the **ggplot2** package (Wickham, 2016). Values
190 are expressed as mean values \pm standard deviations.

191 3. Results

192 3.1. Correlation between HRT and MIB concentration in NP reservoir

193 In total, four significant MIB episodes (peak cMIB $> 30 \text{ ng L}^{-1}$) were observed in NP Reservoir
194 over the period from 2017 to 2020 (Fig. 4). High MIB concentrations were recorded mainly in
195 spring ($27.1 \pm 25.9 \text{ ng L}^{-1}$) and summer ($35.5 \pm 36.1 \text{ ng L}^{-1}$), particularly in April ($48.4 \pm 35.5 \text{ ng}$
196 L^{-1}) and August ($51.3 \pm 55.9 \text{ ng L}^{-1}$), indicating significant seasonality (p -value = 0.0334). The
197 geosmin concentrations remained below the OTC (10 ng L^{-1}) over the entire study period.

198 Limnological characteristics were investigated to explore the potential drivers of MIB episodes,
199 as summarized in Table '!!!TODO KEY ERROR: tbl-NPwqsummaryWAS NOT FOUND!. The mean
200 HRT of NP Reservoir was $19.7 \pm 8.6 \text{ d}$, with higher values in spring ($25.8 \pm 13.8 \text{ d}$), particularly in
201 March ($35.0 \pm 14.0 \text{ d}$) and May ($22.5 \pm 14.1 \text{ d}$), showing significant seasonal variation (p -value
202 = 0.0092). The mean annual water temperature was high and in the range $23.5 \pm 3.3 \text{ }^\circ\text{C}$, with a
203 relatively low temperature difference ($5.7 \text{ }^\circ\text{C}$) between summer ($26.5 \pm 1.3 \text{ }^\circ\text{C}$) and winter (20.8

204 ± 3.7 °C). Nutrients including total nitrogen (TN), total phosphorus (TP), and ammonia (NH₄-N)
205 exhibited no significant seasonal variances (p -values > 0.1), with mean values of $1,502 \pm 401$ μg
206 L^{-1} , 36 ± 30 $\mu\text{g L}^{-1}$, and 152 ± 107 $\mu\text{g L}^{-1}$, respectively.

207 The concentrations of total organic carbon (TOC) were slightly higher in spring (1.65 ± 0.16 mg
208 L^{-1}) and summer (1.76 ± 0.49 mg L^{-1}), showing weak seasonal variance (p -value = 0.0565). The
209 mean annual dissolved oxygen (DO) was 6.9 ± 1.4 mg L^{-1} , exhibiting no seasonal difference (p -
210 value = 0.4690). The phytoplankton abundances were significantly higher (p -value = 0.0084) in
211 spring ($39.7 \pm 38.2 \times 10^6$ cell L^{-1}) and summer ($54.1 \pm 48.8 \times 10^6$ cell L^{-1}), resulting in higher pH
212 values (p -value = 0.0023) of 8.5 ± 0.4 and 8.6 ± 0.5 , respectively.

213 Correlation analysis was performed between the MIB concentration and limnological parame-
214 ters, with only the phytoplankton abundance exhibiting a significantly positive correlation ($r =$
215 0.40 , p -value = 0.0115, Fig. 5). The highest phytoplankton abundance ($(63.6 \pm 49.7) \times 10^6$ cell
216 L^{-1}) occurred in July when the MIB concentration was 38.6 ± 28.0 ng L^{-1} . Moreover, *Pseudan-*
217 *abaena* and *Planktothricoides* were identified as the major MIB-producing cyanobacteria accord-
218 ing to the *mic* gene sequences (Table '!!TODO KEY ERROR: tbl-mibproduceridentificationWAS
219 NOT FOUND!).

220 It should be noted that the MIB concentration and HRT exhibited a similar variation trend (Fig.
221 4), while the change in MIB lagged behind that for HRT by a period of 1 ~ 4 weeks. There was a
222 unimodal distribution between MIB episode probability and HRT, showing that the episode prob-
223 ability was approximately 40% when the HRT was over 17 ~ 20 d (Fig. 6). No significant difference
224 in other major limnological parameters, including temperature, nutrients (TN, TP, NH₄-N, NO₃-
225 N), pH, etc., was observed between the MIB episodes and the other periods (Table '!!TODO KEY
226 ERROR: tbl-wqsignonmibWAS NOT FOUND!).

227 3.2. The effect of HRT on the growth and MIB production of *P. raciborskii* via culture experiment

228 The culture experiment indicated that HRT exhibited a significant impact on the cell growth
229 of the MIB-producing *P. raciborskii*, (p -value < 0.001 , Fig. 1A). Growth was inhibited in low HRT

230 treatments (RT02, RT05), exhibiting significantly lower biomass concentrations in comparison
231 with the other HRT treatments (p -value < 0.0001). Significant differences in growth between
232 moderate (RT10 and RT20) and high HRT treatments (RT40 and RT80) were also observed (p -
233 value < 0.0001). Cell increase lasted for 2 d and 4 d for RT02 and RT05, respectively, and the
234 highest values of $(1.03 \pm 0.49) \times 10^7$ cell L⁻¹ and $(2.02 \pm 0.29) \times 10^7$ cell L⁻¹ were obtained on day
235 3 (RT02) and 5 (RT05), respectively, followed by rapid declines to very low abundance (RT02:
236 0 cell L⁻¹; RT05: $(7.05 \pm 2.44) \times 10^5$ cell L⁻¹) on day 13. Cell increase lasted for 9 d in the RT20
237 treatment, which was 4 d longer than that in the RT10 treatment.

238 MIB concentrations exhibited a similar pattern to the abundance of *P. raciborskii* (Fig. 1B, 1C).
239 The initial MIB concentration was $3,501.0 \pm 295.4$ ng L⁻¹. The MIB concentrations exhibited
240 a clear decreasing trend in RT02 and RT05, which might be associated with evaporation and
241 biodegradation (Li et al., 2012). For RT10 and RT20, the production of MIB during the cell growth
242 period (day 5 ~ 10) could compensate for losses of MIB, and MIB concentrations began to de-
243 crease in the cell decay period. Net increase in MIB concentration was observed only in the
244 RT40 and RT80 treatments. The MIB concentrations in the RT40 and RT80 treatments increased
245 from $3,784.7 \pm 472.2$ and $3,691.7 \pm 343.9$ ng L⁻¹ to $6,838.5 \pm 783.7$ and $15,852.7 \pm 2,644.4$ ng L⁻¹
246 (day 19), respectively. In general, the maximum MIB increase ratio was significantly positively
247 correlated with HRT ($r = 0.76$, p -value = 0.0001).

248 The growth rates (ρ) and maximum cell densities (N^*) were evaluated according to Eq. 3, as
249 summarized in Table 1. Negative growth rate was obtained in RT02 (-0.26 ± 0.14 d^{-1}), while
250 near-zero growth rate was obtained in RT05 (0.02 ± 0.05 d^{-1}). Positive growth rates of $0.19 \pm$
251 0.02 d^{-1} to 0.33 ± 0.11 d^{-1} were obtained for the moderate/high HRT treatments, showing a
252 positive correlation with HRT ($r = 0.65$, p -value = 0.0021). Accordingly, the maximum cell density
253 and MIB concentration were also significantly correlated with HRT ($r = 0.91$, p -value = 0.0107; r
254 = 0.97, p -value = 0.0011).

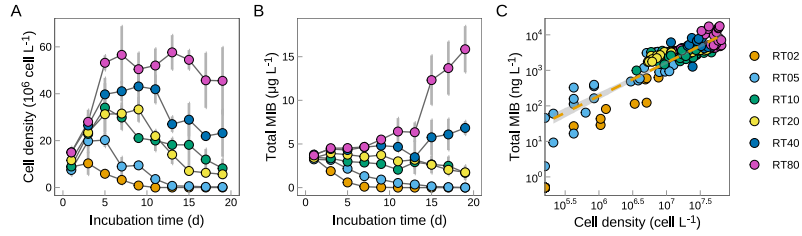


Fig. 1 Cell growth and MIB production of *P. raciborskii* under different HRTs (A: Cell density; B: MIB concentration; C: Correlation between cell density and MIB concentration) (culture condition: temperature: 30 °C, light intensity, 54 $\mu\text{mol m}^{-2} \text{s}^{-1}$; values are present as mean \pm sd)

Table 1 Summary of the *P. raciborskii* culture experiment results (τ (d): the duration of cell number increases; N^* : the maximum cell density; c_{MIB}^* : the maximum MIB concentration; k_{MIB}^* : the decay rates of MIB under different HRT conditions, all values are expressed as mean values \pm standard deviations.)

HRT (d)	τ (d)	ρ (d ⁻¹)	N^* ($\times 10^7$ cell L ⁻¹)	c_{MIB}^* (ng L ⁻¹)	k_{MIB}^* (ng L ⁻¹ d ⁻¹)
2	3	-0.26 \pm 0.14	1.03 \pm 0.48	1,888.7 \pm 96.3	341.1 \pm 19.9
5	5	0.02 \pm 0.05	2.02 \pm 0.29	3,172.1 \pm 197.8	179.4 \pm 7.4
10	5	0.19 \pm 0.02	3.41 \pm 1.04	3,347.8 \pm 334.7	98.6 \pm 45.1
20	9	0.13 \pm 0.10	3.33 \pm 0.52	3,936.9 \pm 82.5	92.3 \pm 5.2
40	9	0.20 \pm 0.11	4.31 \pm 0.75	6,838.5 \pm 783.7	-(178.4 \pm 43.7)
80	13	0.33 \pm 0.11	5.76 \pm 0.73	15,853 \pm 2,644.4	-(675.6 \pm 129.6)

255 The MIB decay rates were 92.3 \pm 5.2 ng L⁻¹ d⁻¹, 98.6 \pm 45.1 ng L⁻¹ d⁻¹, 179.4 \pm 7.4 ng L⁻¹ d⁻¹, and
 256 341.1 \pm 19.9 ng L⁻¹ d⁻¹ for RT20, RT10, RT05 and RT02, respectively. It required 10 d for MIB to
 257 decrease from the initial concentration of 3,410.9 \pm 198.6 ng L⁻¹ (day 1) to 0.5 \pm 0.0 ng L⁻¹ (day
 258 11) in RT02; and 18 d from 3,238.2 \pm 132.9 ng L⁻¹ (day 1) to 9.3 \pm 7.3 ng L⁻¹ (day 19) in RT05.
 259 By comparison, the time required for MIB removal was significantly longer for RT10 (35 d) and
 260 RT020 (37 d).

261 3.3. HRT regulation model

262 The goal of HRT regulation is to reduce the cell increase rate of the targeted cyanobacteria to
 263 less than 0. Here, we propose a model to describe the correlation between the observed cell

264 increase rate (ν , d^{-1}) and HRT (τ , d), as shown in Eq. 3.

$$\nu = \mu_s - \frac{k}{\tau} \quad (3)$$

265 Where μ_s (d^{-1}) and k are undetermined coefficients, defined as the static growth rate and the
 266 hydraulic impact coefficient, respectively. This model indicates that the observed cell increase
 267 rate (ν , d^{-1}) equal to the static growth rate (μ_s , d^{-1}) minus the loss rate caused by the hy-
 268 draulic effects (k/τ). By feeding the culture experiment result of *P. raciborskii*, the model was
 269 determined as $\nu = (0.259 \pm 0.0316) - (1.05 \pm 0.141)/\tau$ ($R^2 = 0.777$, p -value < 0.0001,
 270 Fig. 2A). Accordingly, the critical HRT (τ_L^* , d) for zero growth ($\nu = 0$) was determined to be
 271 $4.1 \pm 1.2 d$ for *P. raciborskii* using k/μ_s . At the same time, the critical HRT values (τ_F^* , d)
 272 for zero MIB increase in two actual MIB episodes were determined to be $7.6 d$ and $6.7 d$, re-
 273 spectively (Fig. 2B, Fig. 2C). Assuming that the static growth rate μ_s was the same for both the
 274 laboratory experiment and the field survey, the hydraulic impact coefficient in the field (k_F) was
 275 determined as $k_F = \tau_F^*/\tau_L^* \cdot k_L$. The critical HRT of two actual MIB episodes were $1.74 \sim 1.97$
 276 times higher than the critical HRT determined by the culture experiment ($4.1 \pm 1.2 d$), implying
 277 that the hydraulic impact coefficient in the field situation (k_F) is $1.83 \sim 2.07$.

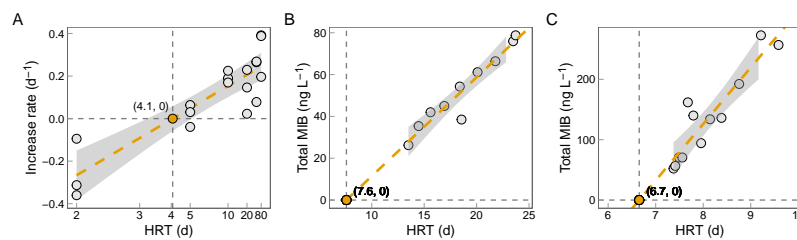


Fig. 2 MIB concentrations are highly correlated to hydraulic retention time according to a culture experiment (A) and two actual MIB episodes occurred in NP reservoir in Zhuhai (B, C)

278 The critical HRT (τ_F^*) determined by k_F/μ_s is the key parameter that can be used to guide the
 279 reservoir operation. The growth rates of 14 cyanobacterial genera based on 1,480 records deter-
 280 mined by culture experiments from references have been summarized in Table '!!TODO KEY ER-
 281 ROR: tbl-growthratefromliteratureWAS NOT FOUND!, and the relationship between the growth

282 rates and critical HRTs for these 14 genera is shown in Fig. 3. Assuming the hydraulic impact co-
 283 efficient in the field situation (k_F) is in the range of 1.5 ~ 3.0, the values of τ_F^* were determined
 284 based on their median static growth rates (μ_s). Higher μ_s requires smaller HRT. For example,
 285 the control of *Synechococcus* requires an HRT shorter than 2.1 ~ 4.2 d, while *Phormidium* can be
 286 well controlled with an HRT shorter than 7.7 ~ 15.4 d. The critical HRT values for frequently
 287 reported MIB-producers including *Planktothrix*, *Planktothricoides*, *Oscillatoria*, *Pseudanabaena*,
 288 *Phormidium* and *Lyngbya* (Izaguirre and Taylor, 2004; Watson, 2003) were determined as 3.4 ~
 289 6.8 d; 4.6 ~ 9.1 d; 5.8 ~ 11.6 d; 7.5 ~ 15.0 d; 7.7 ~ 15.4 d; and 10.3 ~ 20.7 d, respectively (Fig. 3).

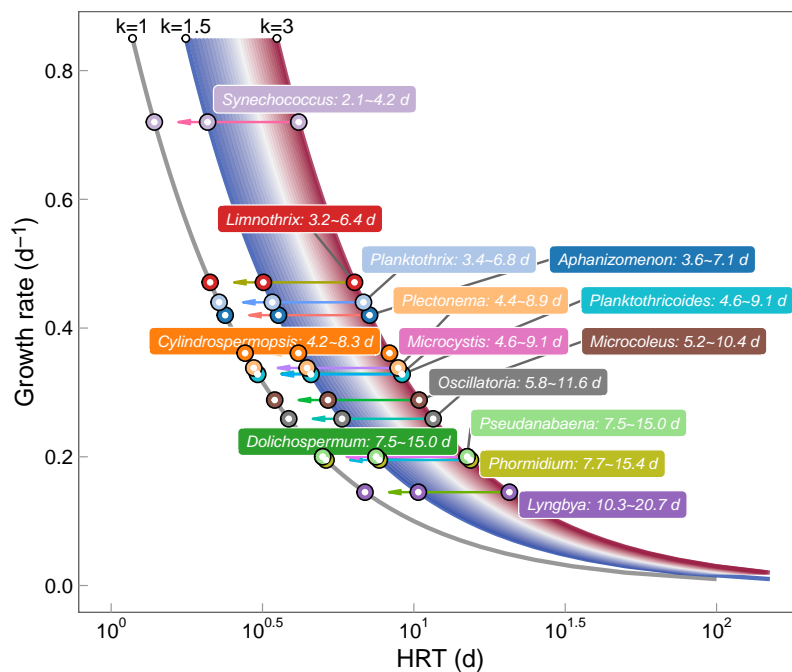


Fig. 3 The critical HRT values τ_F^* for 14 typical cyanobacteria genera determined by the empirical k_F value and their median static growth rates (μ_s)

290 3.4. Field application of HRT regulation for MIB control

291 HRT regulation to impact cyanobacterial growth was applied to ZXD Reservoir in April 2021.
 292 The mean HRT of this reservoir is 12.3 ± 7.3 d, MIB concentration (4.2 ng L^{-1}) and phytoplank-
 293 ton density (chl *a*, $2.5 \text{ } \mu\text{g L}^{-1}$) are relatively low. Nevertheless, it experienced an MIB episode

294 starting on 20 March, 2021, resulting from the suspension of all inflow for 6 *d* from 13 ~ 18
 295 March due to the construction of an upstream pump station. Although the HRT was shortened
 296 to 18.0 ± 2.2 *d* from 19 March, the MIB concentration continued to increase, with the maximum
 297 concentration of 51.7 ± 12.8 ng L⁻¹ observed on 15 April (Fig. 4A). The other main water quality
 298 parameters did not exhibit significant variations during this period (Table ! !TODO KEY ERROR:
 299 tbl-ZXDwqsummaryWAS NOT FOUND!, Table!!TODO KEY ERROR: tbl-ZXDwq2summaryWAS
 300 NOT FOUND!). The mean nutrient concentrations were $1,615 \pm 143$ μg L⁻¹ (TN), 15 ± 3 μg L⁻¹
 301 (TP), 73 ± 59 μg L⁻¹ (NH₄-N) and $1,438 \pm 141$ μg L⁻¹ (NO₃-N), respectively.

302 Cyanobacteria accounted for 91.5% of the phytoplankton community, mainly consisting of
 303 filamentous genera including *Pseudanabaena* (19.8%), *Limnothrix* (29.7%), *Aphanizomenon*
 304 (12.9%), *Cylindrospermopsis* (6.2%), *Dolichospermum* (5.7%) and *Planktothrix* (5.1%), as
 305 illustrated in Fig. 4B. The *Planktothrix* and *Pseudanabaena* were both potential MIB producers
 306 in reservoirs. *Planktothrix* was mostly absent and showed no obvious correlation with MIB (*r* =
 307 -0.58, *p*-value = 0.128). Nevertheless, *Pseudanabaena* exhibited a synchronized increase along
 308 with MIB dynamics from 6.36×10^4 cell L⁻¹ (0.53%, 8 March) to 9.98×10^6 cell L⁻¹ (25.2%, 17
 309 April) (*r* = 0.79, *p*-value = 0.028, Fig. 7). It was thus confirmed to be the main MIB producer in
 310 ZXD Reservoir.

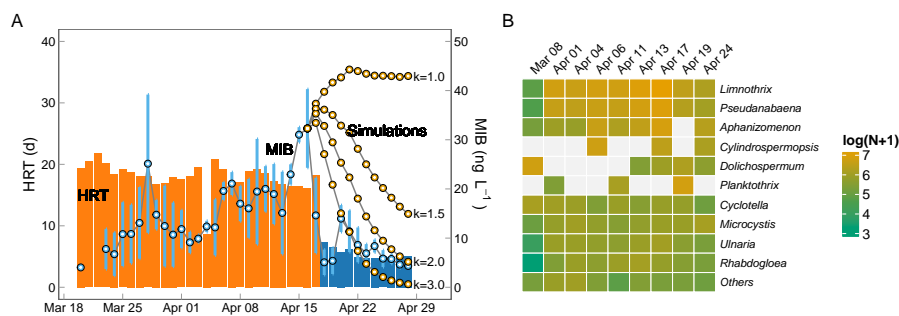


Fig. 4 Variations of MIB concentrations (A) and typical cyanobacterial genera (B) in association with HRT of ZXD Reservoir (the simulated MIB concentrations at different *k*-values are illustrated using black lines with orange circles)

311 On 18 April, HRT regulation was applied by raising the inflow and outflow rates from $10.07 \times$
 312 10^4 to 33.96×10^4 m³ d⁻¹. This resulted in a significant reduction in HRT to 5.4 ± 0.8 *d*, which was

313 below the critical HRT value (τ_F^*) for *Pseudanabaena* (7.5 ~ 15 d, Fig. 4). The k -value was about
314 2.03 (p -value < 0.001) obtained by performing a non-linear regression based on routing data
315 using the **nls** function in R (Fig. 5A). The whole phytoplankton community structure exhibited
316 no significant difference before or after HRT regulation (p -value = 0.072), and was dominated
317 by cyanobacteria with a relative abundance of 93.8 % (Fig. 9). Since the application of HRT
318 regulation, the growth of phytoplankton was inhibited, exhibiting a significant reduction from
319 the maximum observed cell density of 39.61×10^6 cell L⁻¹ (17 April) to 8.86×10^6 cell L⁻¹ (24
320 April). The mean cell density of dominant genera including *Limnothrix*, *Aphanizomenon* and
321 *Cylindrospermopsis* decreased significantly from $(6.98 \pm 4.65) \times 10^6$, $(2.66 \pm 2.61) \times 10^6$, $(1.22 \pm$
322 $2.21) \times 10^6$ cell L⁻¹ to $(2.41 \pm 1.03) \times 10^6$, $(1.12 \pm 1.59) \times 10^6$, $(0.75 \pm 1.06) \times 10^6$ cell L⁻¹ after HRT
323 regulation, respectively. In particular, the MIB producer *Pseudanabaena* exhibited significant
324 reduction in both abundance (from 9.98×10^6 cell L⁻¹ on 17 April to 1.12×10^6 cell L⁻¹ on 24 April)
325 and proportion (from 25.2% to 12.7%). Meanwhile, continuous decrease of MIB was observed
326 from 22.34 ± 4.4 ng L⁻¹ on day 3 of HRT regulation (20 April) to 9.3 ± 2.3 ng L⁻¹ on day 8 (25 April),
327 and remained lower than 10 ng L⁻¹ afterward.

328 Both the phytoplankton abundance and MIB concentration exhibited significant differences
329 between before- and after-HRT regulation. Chl-*a* decreased from an average of 11.3 ± 14.5 μ g
330 L⁻¹ before to 3.7 ± 6.5 μ g L⁻¹ after (p -value = 0.005), particularly for surface and middle layers (p -
331 value < 0.001, Fig. 5A, Fig. 8B). The reduction in MIB concentration between the two periods was
332 also significant (from 22.2 ± 8.7 ng L⁻¹ to 8.6 ± 3.7 ng L⁻¹, p -value < 0.05, Fig. 5B). Simultaneously,
333 several water quality parameters exhibited significant decreases including ammonia, pH, salinity,
334 conductivity, phycocyanin content (PC, Fig. 8C), and ORP (p -values < 0.001, Table '!!TODO KEY
335 ERROR: tbl-ZXDwqcompareWAS NOT FOUND!'). The thermal stratification was weakened (Fig.
336 8A), and TN exhibited a slight increase from $1,563 \pm 97$ μ g L⁻¹ to $1,664 \pm 163$ μ g L⁻¹ (p -value =
337 0.0020).

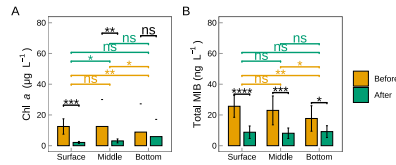


Fig. 5 The variation of Chl-*a* (A) and MIB (B) in different layers before-and-after HRT regulation (ns, not significant, p -value > 0.05; *, p -value < 0.05; **, p -value < 0.01; ***, p -value < 0.001; ****, p -value < 0.0001)

338 4. Discussion

339 4.1. The critical HRT for MIB control in reservoirs

340 By reducing the rate of cell increase (v) to less than 0, the nuisance cyanobacterial population
 341 and their associated water quality problems can be controlled and prevented. Thus, τ_F^* can be
 342 defined as k_F/μ_s by setting v to 0 (Eq. 3), which is determined by the specific growth rate of
 343 the selected cyanobacterial genera (μ_s) and the hydrodynamic regulation coefficient (k_F). The
 344 basis of cyanobacterial control via HRT regulation includes two elements: 1) physical dilution
 345 to prevent cell accumulation or growth, and 2) other physical impacts associated with the side-
 346 effects of hydraulic change. Investigations on river systems have found that shorter HRT can
 347 effectively weaken water thermal stratification, resulting in enhanced vertical mixing and higher
 348 water turbidity (Cha et al., 2017; Mitrovic et al., 2011). This side effect is potentially helpful
 349 for cyanobacterial control, since a well-mixed water column eliminates the cyanobacterial com-
 350 petitive advantage – vertical movement aided by their gas vesicles, over other algae (Huisman
 351 et al., 2018; Walsby et al., 1997; Whitton and Potts, 2007). Moreover, the loss of MIB due to
 352 volatilization may also affect. These additive effects can be represented by the hydraulic im-
 353 pact coefficient (k_F). The coefficient contributed by physical dilution equals 1 according to the
 354 theoretical derivation, while the rest ($k_F - 1$) is contributed by other effects.

355 In this study, the value of k determined in the culture experiment is 1.05 ± 0.141 , suggesting
 356 that the control of *P. raciborskii* is dominated by physical dilution. This is also supported by
 357 another study that reports the effects of disturbance on *Pseudanabaena* growth are limited
 358 (Gao et al., 2018). Nevertheless, the hydraulic impact coefficient in the natural system should

359 be higher than 1 because the aquatic environment is very complex, and many factors are highly
360 correlated to hydraulic conditions (e.g., MIB loss). The hydraulic impact coefficient in NP and
361 ZXD Reservoir (k_F) was 1.83 ~ 2.07, indicating that the removal rate is also contributed by the
362 side effect of HRT regulation (45.3 % ~ 51.7 %) in addition to physical dilution (48.3 % ~ 54.7 %).

363 Considering that k_F is a parameter involving the physical dilution rate ($k_D = 1$) and the side
364 effects of hydraulic change ($k_S = k_F - k_D$), including weakening thermal stratification and
365 increasing turbidity, among others, these side effects are also dependent upon reservoir charac-
366 teristics such as water depth (Yang et al., 2020), water temperature (Wang et al., 2021), inflow
367 characteristics (Sun et al., 2022), and reservoir morphology (Van Breeman and Ketelaars, 1995).
368 Yang et al. (2020) concluded that thermal stratification could be affected by HRT and water depth,
369 according to an investigation of eight karst hydropower reservoirs in Wujiang River basin in China.
370 The turbidity in reservoir water is also affected by water temperature and inflow characteristics
371 (Sun et al., 2022; Wang et al., 2021). Moreover, Van Breeman and Ketelaars (1995) declared that
372 the mixing characteristics in reservoirs depend upon their morphology, which determines the
373 effectiveness of cyanobacterial control. Thus, we believe that k_F is almost a constant for a spe-
374 cific reservoir and suggest that k_F should be adjusted when applied to actual reservoirs. In this
375 study, it was estimated from the MIB episodes in the NP and ZXD Reservoirs, and we empirically
376 propose that k_F can be given a value in the range of 1.5 ~ 3.0 if no specific evidence is available.

377 The specific growth rate (μ_s) varies among different cyanobacterial genera (Jia et al., 2019; Lu
378 et al., 2022; Wang and Li, 2015), and genera with higher μ_s require a shorter critical HRT (τ_F^*)
379 to limit their cell increase (Fig. 3). Nevertheless, the growth rate of cyanobacterial cells is also a
380 function of environmental factors such as light intensity, temperature, nutrients, etc. (Jia et al.,
381 2019; Lu et al., 2022; Su et al., 2021b), so the critical HRT is different in different seasons and/or
382 different reservoirs. This is most likely the reason for the 0.9-day difference in critical HRT values
383 (6.7 d and 7.6 d) between the two MIB episodes in NP Reservoir. To simplify model Eq. 3, the
384 median values of the cyanobacterial genera growth rates reported in literature were used in this
385 study to calculate critical HRTs (τ_F^*) with different k_F values (Fig. 3). These values can provide a
386 grounded basis for HRT regulation for MIB control in drinking water reservoirs, and they can also

387 help understand phytoplankton community composition in various water bodies with different
388 HRTs. Nevertheless, although we have tried our best to review all available literature regarding
389 the growth rates of typical cyanobacteria, the growth rates are not necessarily accurate enough
390 and may result in imprecise critical HRT values.

391 4.2. Application of HRT regulation in drinking water reservoirs

392 It is useful to compare the growth potential of more widespread and typical surface bloom-
393 forming cyanobacteria genera such as *Microcystis*, *Aphanizomenon*, *Cylindrospermopsis*, etc., to
394 the MIB-producing genera. The latter tend to have relatively lower specific growth rates ($0.09 \sim$
395 0.39 d^{-1} , Table [!!TODO KEY ERROR: tbl-growthratefromliteratureWAS NOT FOUND!](#)), which sug-
396 gests that regulation based upon shorter HRT is potentially more effective for controlling these
397 MIB-producing genera. In the case reported here, HRT regulation applied in ZXD Reservoir suc-
398 cessfully controlled the MIB problems in 2021 (Fig. 4). During the MIB episode, the HRT thresh-
399 old of the MIB producer *Pseudanabaena* was determined as $7.5 \sim 15.0 \text{ d}$ based on 69 records
400 from literatures (Fig. 3), which was shorter than the HRT of ZXD Reservoir ($18.0 \pm 2.2 \text{ d}$) before
401 the application of HRT regulation. In comparison, the HRT was lowered to $5.4 \pm 0.8 \text{ d}$ by raising
402 the flow rate after 18 April, resulting in significant decreases in *Pseudanabaena* abundance and
403 MIB concentration. The fitted k -value in ZXD Reservoir was about 2.03 (Fig. 4A), indicating that
404 the critical HRT was $10 \sim 15 \text{ d}$ and consistent with our empirically proposed k_F value ($1.5 \sim 3.0$).
405 Besides, both Chl-*a* concentration and phytoplankton cell density were markedly decreased af-
406 ter regulation (Fig. 5A, Fig. 4B). These results support that HRT regulation can be used to control
407 this type of nuisance cyanobacteria and their related water quality problems. Nevertheless, the
408 growth rate of *Pseudanabaena*, that was not determined by specialized culture experiment, may
409 result in imprecise evaluation of HRT threshold.

410 Investigations into other strategies to control harmful cyanobacteria in reservoirs have shown
411 clear evidence of influence on the structure of phytoplankton communities (Burford and
412 O'donohue, 2006; Moustaka-Gouni et al., 2022; Wan et al., 2021). Wan et al. (2021) found a
413 distinct variation in bacterioplankton community composition caused by changes in total phos-

414 phorus after dredging. [Lusty and Gobler \(2020\)](#) found a significant reduction in cyanobacteria
415 biomass after hydrogen peroxide was added, but the sensitivity of different genera varied.
416 [Burford and O'donohue \(2006\)](#) declared that artificial mixing was more effective in controlling
417 colonial versus solitary filamentous species and promoted the growth of species with low
418 phosphorus tolerance. By contrast, in this study, the phytoplankton community structure was
419 not significantly affected by HRT regulation (p -value = 0.072, Fig. 4B). The HRT regulation principle
420 includes physical dilution ($1/\tau$), which was consistent for the effect on all genera; while
421 specific growth rates varied among different genera, and typical bloom-forming cyanobacteria
422 genera with higher growth rates and lower critical HRTs could gain growth advantages over
423 others under shorter HRT conditions, as also suggested by [Robarts and Zohary \(1987\)](#). In ZXD
424 Reservoir, the mean *Pseudanabaena* cell abundance was drastically reduced by 65.4% after
425 HRT regulation, whereas some bloom-forming genera with higher growth rates, including *Aph-*
426 *anizomenon* and *Cylindrospermopsis*, descended to relatively lower values at about 57.8% and
427 38.7%, respectively (Fig. 4B). Therefore, by adjusting the HRT the phytoplankton structure can
428 potentially be modified towards a preferred community structure with less abundant harmful
429 algae. It is worth noting that HRT-based regulation to control MIB-producing cyanobacteria
430 is more suitable for small drinking water reservoirs, especially in areas with well-developed
431 water systems but cyanobacteria-derived MIB problems are frequent including South China
432 and South East Asia, etc. ([Lu et al., 2022](#)). In these areas, large number of small reservoirs
433 have been built for flood control, drought, water supply and irrigation, making it easier to
434 control odor by reducing HRT through mutual water transfers. Nevertheless, shortening HRT in
435 medium/large reservoirs can cause significant water and energy consumption. Additionally, this
436 strategy is also limited by the fact that the odor compounds or other contaminants (nutrient,
437 phytoplankton, etc.) in inflow water should be at low level. Besides, the higher flushing rates
438 associated with shorter HRTs are more suitable for those planktonic cyanobacterial genera
439 control, while other strategies (water level and turbidity regulation, etc.) are more effective
440 for reservoirs with benthic/deep-living genera ([Jia et al., 2019](#); [Su et al., 2017](#)). The hydraulic
441 regulation may not be able to deal with the MIB problem caused by actinomycetes and other

442 microorganisms in source water reservoirs, and therefore further investigations are required.

443 **5. Conclusion**

444 This study carried out field investigations, laboratory culture experiments, and field applica-
445 tions in response to frequently observed MIB episodes in drinking water reservoirs in China. The
446 following conclusions could be drawn: 1) Increases in HRT can trigger MIB-derived odor prob-
447 lems in reservoirs; 2) The maximum cell density, growth rate, and MIB concentration exhibited
448 significant positive correlations with HRT according to the culture experiment of MIB-producing
449 *P. raciborskii*, and growth was greatly inhibited when HRT was less than 5 *d*; 3) A HRT regula-
450 tion model that aims to control harmful cyanobacteria and associated water quality problems
451 was developed, and the critical HRT values for controlling typical cyanobacterial genera were
452 evaluated; 4) HRT regulation was successfully applied by decreasing the HRT to lower than the
453 critical value in ZXD Reservoir, resulting in a significant decrease of MIB concentration. The pro-
454 posed principle provides a scientific basis for HRT regulation strategy and can be used to control
455 MIB-derived odor problems, and this should also work for the control of other MIB-producing
456 cyanobacteria.

457 **Declaration of Competing Interest**

458 The authors declare that they have no known competing financial interests or personal rela-
459 tionships that could have appeared to influence the work reported in this paper.

460 **Acknowledgement**

461 This work was financially supported by the National Natural Science Foundation of China
462 (52030002, 51878649), China Key Research and Development Program (2022YFC3203603) and
463 Youth Innovation Promotion Association CAS.

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