The effect of oxidants on 2-MIB concentration with the presence of cyanobacteria

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Abstract In this study, the effect of three oxidants, sodium hypochlorite, potassium permanganate, and ozone, were tested for the removal of 2-MIB with presence of cyanobacteria. Algae in water samples from the source water of Feng-Shen waterworks (FSW), Taiwan were cultivated at 30°C with continuous light at an intensity between 2,500 and 3,400 lux. During the cultivating process, water samples were analyzed for nutrients, light absorbance at 665nm (A665), and 2-MIB concentration. The 2-MIB concentrations within the incubated samples increased to as high as 1,000 ng/L to 2,000 ng/L, although no extra nutrients were added to the raw water. After 2 to 3 days incubation, the intracellular 2-MIB concentration was as high as 70% of the total 2-MIB in the samples. The algae that developed were mainly cyanobateria, and more than 90% belonged to the Genus *Oscillatoria*s. An almost 100% removal of both 2-MIB and geosmin in the raw water was observed after ozonation for 10 minutes at a dosing rate of 0.91 mg/l-min. Chlorine and permanganate were much less effective, both removing only about 11% of the 2-MIB within 60 minutes at oxidant concentration of 10 mg/l. Oxidation of the cultivated samples showed that chlorine and permanganate may damage algae cells causing them to release intracellular 2-MIB. During the 60 minutes of reaction time, the total 2-MIB concentrations (intracellular plus dissolved) varied by no more than 10%, however, the ratios between dissolved and total 2-MIB concentrations increased. Two effects of ozonation on the 2-MIB concentration in the cultivated samples were observed when the algae were young, namely 2-MIB release from damaged cells and 2-MIB oxidization. The rates of 2-MIB release and 2-MIB destruction were similar. However, old algae cells were more easily damaged. As a result, intracellular 2-MIB was released faster, and the soluble 2-MIB was destroyed more quickly by ozonation.

Keywords Cyanobacteria; drinking water; geosmin; 2-MIB; oxidation

Introduction

Musty odor is present in the source water of Feng-Shen waterworks (FSW), Taiwan year round. A 2-MIB concentration of between 10 ng/L and 130 ng/L was normally detected in the source water (Lin *et al*., 2002a). Although the populations of phytoplankton and actinomycetes have been linked to the strength of musty odors in the area, the microorganism responsible for the musty odors remains unknown. Two oxidants, chlorine and permanganate are often used in Taiwan's waterworks for the control of ammonia and odors. In addition, ozonation will be used to replace the pre-chlorination process in a few of the waterworks in Taiwan soon. One problem associated with the use of oxidation in the raw water is that 2-MIB within the algal cells may be released into the water. Although 1/2 to 2/3 of 2-MIB have been found to be retained in the cells of *Oscillatoria* sp. (Rashash *et al*., 1995), the relative quantity of 2-MIB in the cells to that in the water for the source water of FSW needs to be understood. In addition, the effect of oxidants on the reduction and/or release of 2-MIB from the microorganism also needs to be known.

In this study, the 2-MIB generating microorganisms were first cultivated in the laboratory. During the cultivation, water samples with and without filtration were analyzed

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for the 2-MIB concentration. The filtered sample represents a measurement of the dissolved 2-MIB, while the sample without filtration represents a summation of dissolved and intracellular 2-MIB (within the algae cells). To understand the effect of oxidants on the 2-MIB concentration with the presence of microorganism, the cultivated water samples with algal blooms were then used in the oxidation experiments. Three oxidants, sodium hypochlorite, potassium permanganate, and ozone were tested.

Experimental

Chemicals

The analytical standard for 2-MIB was purchased from Sigma (analytical grade, Germany) at a concentration of 2 mg/L, while that for geosmin was obtained from Wako (analytical grade, Japan) at 0.1 mg/L. Sodium hypochlorite from Riedel-de Haen (6–14% solution, Extra Pure, Germany) was used to simulate free chlorine in the laboratory tests, while sodium thiosulfate from Baker (Pentahydrate form, Reagent Grade, USA) was used to remove the oxidants in the samples.

Cultivation of cyanobacteria

Water samples from the source water of Feng-Shen Waterworks (FSW) were placed into 5-L glass reaction vessels and maintained at 30 ± 0.5°C in a temperature-controlled cabinet. No extra nutrients were added into the samples. The light intensity in the cabinet was controlled at between 2,500 lux and 3,400 lux, and air was bubbled through the vessels at 1–2 ml/min. Sampling and analysis of the cultivated samples was conducted once a day after incubation. After 3 to 5 days, the algae population had increased to bloom proportions, and 2-MIB concentrations increased to several hundred ng/l. The predominant algae were cyanobacteria, and the major Genus was Oscillatoria at around 2×10^4 trichomes/ml counting with a Sedgwick-Rafter Counting Cell as prescribed in *Standard Methods* (APHA *et al.*, 1998).

Ozonation experiments

A semi-batch type reactor was employed in the ozonation experiment, in which gaseous ozone is continuously charged into the reactor with water sample. No water flowed into and out from the reactor. The reactor, with a volume of 2.2 l, was maintained at constant temperature (25 ± 1°C). Both the source water of FSW and laboratory-cultivated water were used in the experiments. The FSW source water was used as collected, while the cultivated water was taken from the incubated samples (see Cultivation of Cyanobacteria section) at 4, 5 and 6 days. In the experiment, light absorbance at 665 nm, and total and soluble 2-MIB were analyzed at 1, 2, 5 and 10 minutes. An ozone generator (Fischer, Model Oz 500, Germany) was used in the study. The dosing rate of ozone into the reactor was controlled at 0.91 mg/l-min.

Chlorine and permanganate oxidation experiments

One-litre batch reactors were used for the evaluation of chlorine and potassium permanganate oxidation. The reactors, maintained at 25 ± 1°C, were filled with cultivated water and stirred continuously with a Teflon-coated magnetic stirrer within the reactor. The incubated FSW water at day 3 of cultivation was used in the oxidation experiments of chlorine and permanganate. The dosage of chlorine and permanganate were controlled at 2 mg/l and 10 mg/l. The contact times were set at 10 to 60 minutes. Again, light absorbance at 665 nm, and total and soluble 2-MIB were monitored.

Analysis

Gas-phase ozone concentrations were determined by Ozone Demand/Requirement: Semi-Batch Method, while dissolved ozone was determined by the Indigo Colorimetric Method, both reported in *Standard Method for the Examination of Water and Wastewater* (APHA *et al*., 1998). A Water Quality Analyzer (Merck, Nova 60, USA) was used to determine the concentration of residual chlorine, ammonium nitrogen, nitrate, nitrite, and phosphate. Permanganate concentration was determined by the Spectrophotometer Method in the *Standard Methods* (APHA *et al*., 1998).

The concentrations of 2-MIB and geosmin were analyzed using the solid-phase microextraction (SPME) coupled with a gas chromatograph (GC) and a mass spectrometer (MS). A commercially available fiber (30/50 μm DVB/CAR/PDMS (No. 57348-U)) coupled with a manual fiber holder (No. 57330-U), both from Supelco (Bellefonte, PA, USA), were selected for the extraction of 2-MIB and geosmin in some cases. A GC/MS from Hewlett-Packard (USA) (HP-6890 and HP-5973) was employed for the analysis. The procedure for the analysis of 2-MIB and geosmin was the same as that prescribed by *Standard Methods* 6040D (APHA *et al*., 2000), except for some minor differences. Detailed information regarding the operation of analysis was previously reported by Lin *et al*. (2002b). The calibration curves for both 2-MIB and geosmin were established between 2 ng/L and 200 ng/L, with high linearity and high regression coefficients ($R^2 > 0.998$). Differentiation between soluble 2-MIB and 2-MIB in the algal cells was determined by separate analyses of filtered and unfiltered samples. A 0.7 μm glass fiber filter (Whatman, GF/F, USA) was used for the separation.

Estimates of the algal population densities in the laboratory cultures were determined by chlorophyll a analysis. The samples were first filtrated through a 0.70 μm glass fiber filter (Whatman, GF/F, USA), and chlorophyll a was extracted from the solid on the filter with ethanol (99.8% purity, Ferak, Germany). The filtrate absorbance at 665 nm was then determined. Although the method does not provide a precise quantification of the algae mass, it does reflect the growth status of algae.

Results and discussion

2-MIB concentration in the cultivated water

Indigenous algae in the raw water from FSW were cultivated at two different time periods, one in April 2002 (dry season) and one in September 2002 (wet season). Figure 1 illustrates the concentration change of nutrient species and 2-MIB in the cultivated water at different times. The 2-MIB concentrations (Figure 1(b) and 1(d)) increased to 1,000 ng/l to 2,000 ng/l even though nutrients were not added into the system. The quantity of intracellular 2-MIB was as much as 70% of the total 2-MIB in the samples. The percentage of MIB found within the cells in this organism is comparable to that reported by Rashash *et al*. (1995), which is from 50% to 66%. The total nitrogen contents were similar for the two samples, but different in the initial form of nitrogen. The ammonia concentration in first run has relatively high (4.3 mg/l), and the nitrate concentration relatively low (2.5 mg/l), while the ammonium concentration during the second run was low (0.9 mg/l) but the nitrate concentration relatively high (7.6 mg/l). These differences in nutrient concentration influenced the growth of algae as well as the production of 2-MIB. For example, there was a one to two day time lag before the 2-MIB began to increase during run 1, but almost no lag period was observed during run 2. The highest 2-MIB concentration was observed at day 7 during run 1 and at day 5 during run 2. The difference may be attributed to the fact that the form of nitrogen (ammonium ion) during run 1 was not the optimal nitrogen source for the algae. In fact, nitrate is the nitrogen source in several common cyanobacteria growth media, such as BG-11 (Rippka *et al.,* 1979). As illustrated in Figure 1(a), almost two days were required for the ammonium ion to be fully oxidized to the usable form of nitrogen (nitrate).

As shown in Figure 1(b) and 1(d), most of the 2-MIB in the raw water was in dissolved form. During the first 3 to 4 days, concentrations of total and intracellular 2-MIB increased at similar rates, which indicates that most of the 2-MIB remained within the cells. The percentage of dissolved 2-MIB increased significantly with increasing culture age. After day 7, 2-MIB concentration declined and this may be due to bacterial degradation. Increase in culture pH and absorbance (A665) shown in Figure 1(c) and 1(d) are both indicators of algae growth. The observation that the healthier cells tend to keep 2-MIB within the cells is similar to that reported by Rashash *et al*. (1995). In addition, release from and retention in algal cells for 2-MIB and geosmin were also reported elsewhere (Wu and Jüttner, 1988; Rosen *et al*., 1992). Wu and Jüttner (1988) observed that geosmin and MIB were retained in the cells for *Oscillatoria tenuis*, rather than in the growth medium, and Rosen *et al*. (1992) concluded that the stationary phase of *Anabaena circinalis* caused the release of cell associated geosmin into the medium.

2-MIB removal from raw water

Figure 2 shows the removal ratio of 2-MIB in the FSW raw water by the three oxidants. Since most of the 2-MIB (in this case, geosmin is also reported) was present in dissolved form, it is a representation for the reaction without the presence of algae. The reaction kinetics of the ozone/2-MIB and geosmin system was almost linear as shown in Figure 2(a). The removal efficiencies for both 2-MIB and geosmin were very high, and only 10 minutes were needed for almost complete oxidation of both odorants. Glaze *et al*. (1990) reported similar results in which 80–90% of geosmin and 2-MIB were removed after 20 minutes at ozone dosing rate of 0.2 mg/L-min.

Figure 2(b) shows that only about 11% of 2-MIB was oxidized by 10 mg/L permanganate in 60 minutes, and chlorine was even less effective (<5% reduction). These data are similar to those of Glaze *et al*. (1990). The geosmin removals during oxidation with chlorine and permanganate are not shown in Figure 2(b) because the initial concentration was quite low and the removal was insignificant. Lalezary *et al*. (1986) observed that neither chlorine nor permanganate was effective to destroy 2-MIB and geosmin, which is similar to our experimental results.

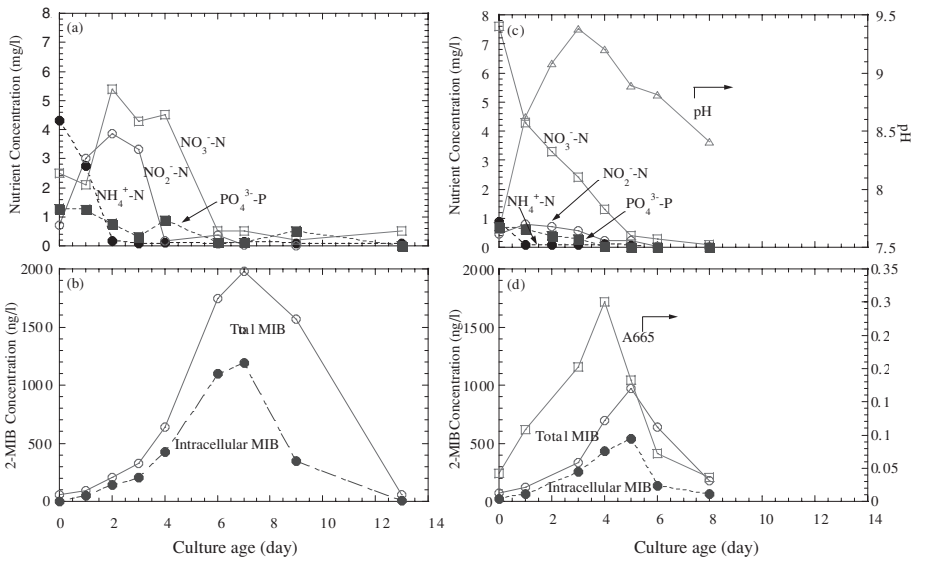

Figure 1 The concentration change of nutrient and 2-MIB in two cultivated experiments, where (a) and (b) were samples from dry season, and (c) and (d) were samples from wet season

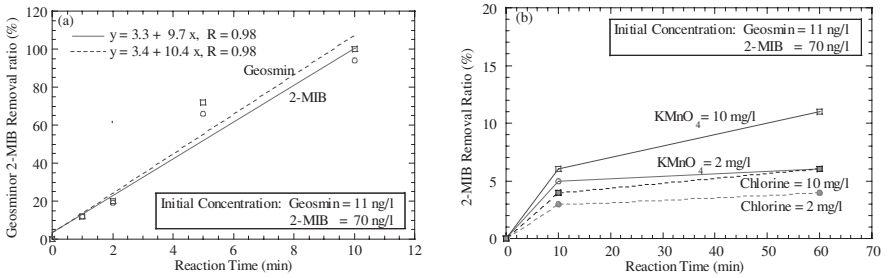

Figure 2 Oxidation kinetics of 2-MIB in unfiltered FSW raw water by (a) ozone, and (b) chlorine and potassium permanganate

Chlorine and permanganate oxidation of cultivated water

Since the oxidation efficiency of 2-MIB by the two chemicals was very small, as shown in Figure 2(b), no significant removal of 2-MIB was expected. However, the data clearly show that soluble 2-MIB concentration increased following addition of the oxidants. Most likely, chlorine and permanganate damaged the algae cells causing release of intracellular 2-MIB into the water. Ashitani *et al*. (1988) observed an increase of 2-MIB and geosmin concentrations in water following prechlorination at a water treatment plant. During the 60 minutes reaction time, the total 2-MIB concentrations (intracellular plus dissolved) remained almost constant (within 10% of difference). But the ratio between dissolved and total 2-MIB concentrations increased markedly (Figure 3). Most of the intracellular 2-MIB was released within 10 minutes after chlorine was added at two different doses (Figure 3(a)). The effects of permanganate appeared more slowly, and significant release occurred after 10 minutes only when the dose was 10 mg/l (Figure 3(b)). Our observation is similar to that reported in the literature (Lam *et al*., 1995; Peterson *et al*., 1995). Lam *et al*. (1995) indicated that cyanobacterial cells might be lysed in the presence of chlorine and permanganate. Peterson *et al*. (1995) also found that chlorine and permanganate caused extensive damage of *Aphanizomenon flos-aquae* cells, inducing the release of geosmin and dissolved organic carbon.

Figure 3(a) also illustrated the changes in sample absorbance at 665 nm, which is one of the wavelengths used in the spectrophotometric method for chlorophyll a analysis of maximum absorbance for chlorophyll a (APHA *et al*., 1998). Although most of the 2-MIB was released into the water within 10 minutes, the absorbance, and, by inference, the chlorophyll a concentration, did not change substantially (only 30% reduction within 10 minutes at most). Also observed is a much greater reduction of light absorbance at 665 nm in the system for chlorine at 10 mg/l than that at 2 mg/l. Similar observation was also found in the next section (Ozonation of Cultivated Water).

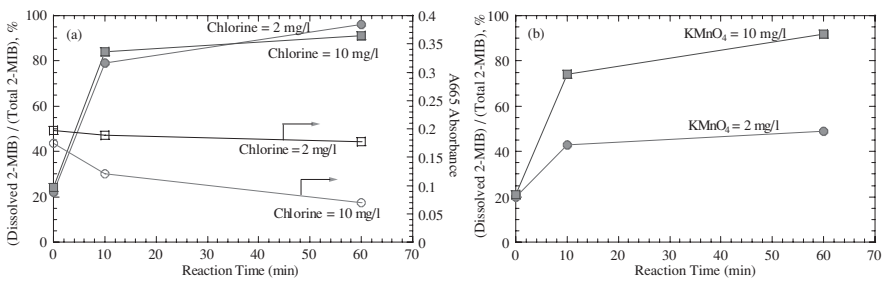

Figure 3 Oxidation kinetics of 2-MIB in the cultivated water by (a) chlorine and (b) potassium permanganate

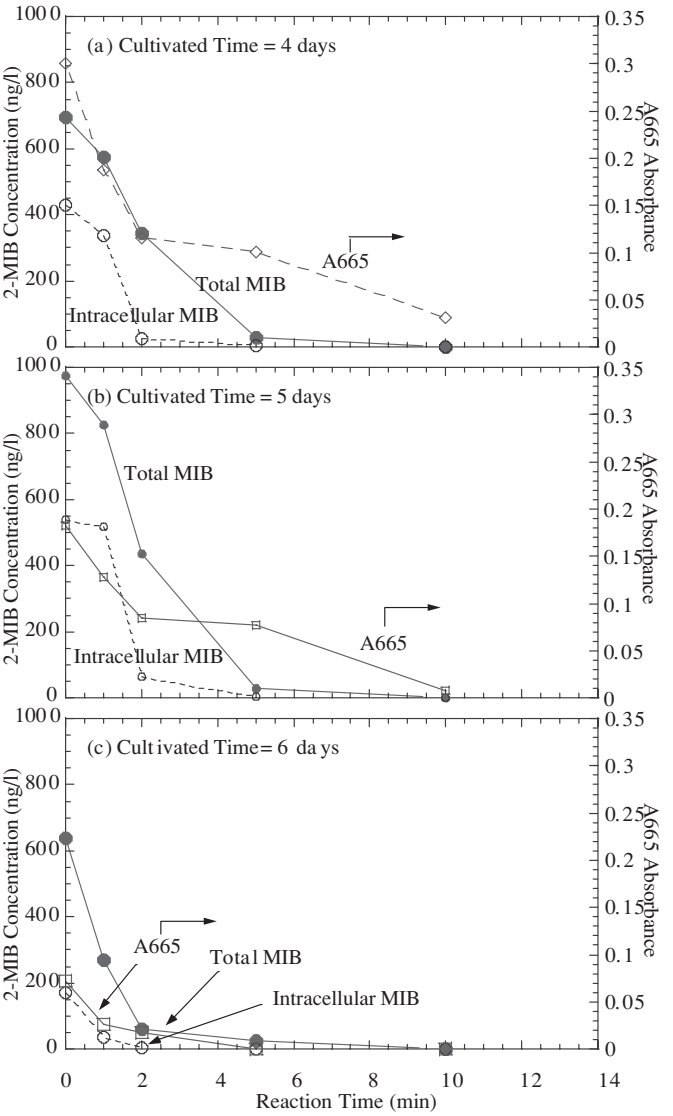

Figure 4 Oxidation kinetics of 2-MIB by ozone using the FSW source water cultivated for (a) 4, (b) 5, and (c) 6 days

Ozonation of cultivated water

Ozone was evaluated in three experiments. Samples of the incubated raw water were collected after 4, 5, and 6 days of incubation representing three of the typical growth stages of the culture shown in Figure 1(d) (day 4, growth phase; day 5, stationary phase; and day 6, decay phase). The effects of ozonation on the 2-MIB concentrations may be categorized into two types: one is cell damage causing the release of 2-MIB, and the other is the destruction of dissolved 2-MIB. Initially, about 50% of 2-MIB was in the cells in samples collected on day 4 (Figure 4(a), referred to as Case (a)) and day 5 (Figure 4(b), Case (b)), and only 20% was present in the cells in the sample collected on day 6 (Figure 4(c), Case (c)). Figure 4(a) and 4(b) show that the total 2-MIB concentration declined at approximately the same rate as the intracellular 2-MIB during first two minutes (Cases (a) and (b)), which may be attributed to the combined effect of 2-MIB release from damaged cells and 2-MIB oxidized by ozonation. However, this was not the same situation for Case (c) and in samples

ozonated for longer periods in (a) and (b). The cells in these cases were more easily damaged, and thus, release of intracellular 2-MIB and destruction of aqueous phase 2-MIB occurred faster.

It was also observed that the younger (and by inference healthier) cells (Figure 4(a)) released 2-MIB more slowly than the older, decaying cells (Figure 4(c)). A similar trend was also observed in the reduction rate of absorbance at 665 nm. These rate differences (2-MIB release and chlorophyll a reduction) may be attributed to the greater susceptibility of the older cells to the oxidant.

Summary and conclusions

Most of the algae that developed in FSW raw water during the incubation period in the laboratory were cyanobateria, and more than 90 % of these belonged to the Genus *Oscillatoria*. The 2-MIB concentrations increased to 1,000–2,000 ng/l after 5–7 days of incubation and as much as 70 % of the 2-MIB in the water was present within the algae cells. Ozonation of the raw water for 10 minutes at a dosing rate of 0.91 mg/l-min removed almost 100% of both 2-MIB and geosmin. In contrast, chlorine and potassium permanganate (10 mg/L) removed only about 11% of the 2-MIB from the raw water. Oxidation of incubated samples with chlorine and permanganate caused the cell damage and release of intracellular 2-MIB. On the other hand, ozonation of the incubated water samples appeared to affect the 2-MIB concentrations in two ways: (1) caused the release of 2-MIB from damaged cells and (2) oxidized soluble 2-MIB. When the algae cells were young, the rates of 2-MIB release and destruction were approximately the same, but when the algae culture was in the decay phase, the cells were more easily damaged, thus causing a greater release of intracellular 2-MIB and a faster rate of destruction of soluble 2-MIB. Based on the observation of this research, neither chlorine nor permanganate is appropriate for the oxidation of a eutrophic raw water like FSW. Instead, to reduce the release of 2-MIB into water, 2-MIB-laden algae should be removed before contacting with the oxidants. On the other hand, ozonation is effective in the destruction of both soluble and intracellular 2-MIB. However, it may damage algal cells and cause the increase of dissolved organic compounds in water.

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References

APHA, AWWA, and WPCF (1998). *Supplement to Standard Methods for the Examination of Water and Wastewater*, 20th ed., Denver, Colorado, USA.

APHA, AWWA, and WPCF (2000). *Supplement to Standard Methods for the Examination of Water and Wastewater*, 20th ed., Denver, Colorado, USA.

Ashitani, K., Ishiba, Y. and Fujuwara, K. (1988). Behavior of musty odorous compounds during the process of water treatment. *Wat. Sci. Tech.*, **20**(8/9), 261–267.

Glaze, W.H., Schep, R., Chauncey, W., Ruth, E.C., Zarnoch, J.J., Aieta, E.M., Tate, C.H. and McGuire, M.J. (1990). Evaluating oxidants for the removal of model taste and odor compounds from a municipal water supply. *J. AWWA*, **82**, 79–84.

Lalezary, S., Pirbazari, M. and McGuire, M.J. (1986). Oxidation of Five Earthy-Musty Taste and Odor compounds. *J. AWWA*, **78**(3), 62–69.

Lam, A.K.Y., Prepas, E.E., Spink, D. and Hrudey, S.E. (1995). Chemical control of hepatotoxic phytoplankton blooms: implications for human health. *Wat. Res.*, **29**(8), 1845–1854.

Lin, T.F., Wong, J.Y. and Kao, H.P. (2002a). Correlation of musty odor and 2-MIB in two drinking water treatment plants in south Taiwan. *Sci. Total Environ.*, **289**, 225–235.

Lin, T.F., Liu, C.L., Yang, F.C. and Hoang, S.W. (2002b). Effect of residual chlorine on the analysis of geosmin, 2-MIB and MTBE in drinking water using the SPME technique. *Wat. Res.*, **37**(1), 21–26.

Peterson, H.G., Hrudey, S.E., Cantin, I.A., Perley, T.R. and Kenefrick, S.L. (1995). Physiological toxicity, cell membrane damage and the release of dissolved organic carbon and geosmin by *Aphanizomenon flos-aquae* after exposure to water treatment chemicals. *Wat. Res.*, **29**(6), 1515–1523.

Rashash, D.M.C., Dietrich, A.M., Hoehn, R.C. and Parker, B.C. (1995). The influence of growth condition on odor-compound production by two chrysophytes and two cyanobacteria. *Wat. Sci. Tech.*, **31**(11), 165–172.

Rosen, B.H., Macleod, B.W. and Simpson, M.R. (1992). Acculation and release of geosmin during growth phase of *Anabaena Circinalis (Kutz) Rabenhorst. Wat. Sci. Tech.*, **25**(2), 185–190.

Wu, J.T. and Jüttner, F. (1988). Differential partitioning of geosmin and 2-methylisoborneolbetween cellular constituents in Oscillatoria tenuis. *Archiv. Microbiol.*, **150**, 580–583.