Biodegradation of MIB, geosmin and microcystin-LR in sand columns containing Taihu lake sediment
Stephanie L. DeVries, Wenjie Liu, Neng Wan, Pengfei Zhang and Xiqing Li

ABSTRACT
Lake Taihu, located in southeast Jiangsu Province, China, is an important drinking water resource serving almost 2 million residents nearby. In recent years, eutrophication has led to more frequent cyanobacterial algae blooms whose metabolites, including 2-methylisoborneol (MIB), 1,10-dimethyl-trans-9-decalol (geosmin) and microcystin-LR (MC-LR), can be both toxic and unpalatable. Traditional treatment methods are expensive and often poorly remove these compounds. Since biodegradation has shown promise, the removal of MIB, geosmin and MC-LR in columns containing a mixture of quartz sand and Taihu lake bed sediment was tested. Results show that 24, 38.5 and 100% removal of MIB, geosmin and MC-LR, respectively, can be achieved at a 1 m d$^{-1}$ flow rate through 30 cm long columns containing 10% Taihu sediment. Biodegradation rates derived from one-dimensional transport modeling were used to predict the performance of 2 m filtration beds of similar composition. The results indicate that satisfactory removal of MIB, geosmin and MC-LR can be achieved by this method.

Key words | biodegradation, geosmin, infiltration gallery, methylisoborneol, microcystin

INTRODUCTION
Lake Taihu, located on the border between Jiangsu and Zhejiang Provinces, is the third-largest fresh water lake in China, with a surface area of approximately 2,400 km$^2$. Surrounded by six large cities, Taihu is an economically important fishery and a primary drinking water resource serving over 2 million people. Economic development in this region has flourished in the last few decades, driving a concomitant increase in water pollution and usage, thus rendering the lake prone to eutrophication and seasonal development of large cyanobacterial algae blooms (Zhang & Qin 2001) whose metabolites can be toxic and unpalatable. Microcystins (MCS), produced by *Microcystis aeruginosa* and other blue-green algae, are a class of cyclic hepatotoxins whose most common variant, microcystin-LR (MC-LR), has been linked to both chronic and acute health risks, including tumor growth (Carmichael 1994), high rates of primary liver cancer (Yu 1995) and possibly death (Lambert et al. 1994). Though non-toxic, two common taste and odor compounds, 2-methylisoborneol (MIB) and 1,10-dimethyl-trans-9-decalol (geosmin), are also produced by a range of cyanobacterial species. Each imparts a foul earthy/musky odor and flavor to water (Carmichael 1992) and are detectable by the human nose at concentrations as low as 2–10 ng L$^{-1}$ (Per-Edvin 1980). The presence of MIB and geosmin in drinking water can give the impression of poor quality to consumers, who subsequently seek alternative water supplies, often at higher consumer and environmental cost (Srinivasan & Sorial 2011). In order to prevent a drinking water crisis in the Taihu region, a low-cost and effective method is needed to remove MC, MIB and geosmin before it reaches the tap.

Traditional water treatment methods (e.g., coagulation, filtration) poorly remove dissolved MC-LR, MIB and geosmin (Bruce et al. 2002; Svrcek & Smith 2004). Powdered activated carbon (PAC) has proven reasonably effective at removing both MIB and geosmin, but the required dosage for complete removal varies with influent concentrations, type of activated carbon used and competition for sorption sites from natural organic material (NOM) (Lalezary et al. 1986; Huang et al. 2007), so optimization can be challenging. Similarly, PAC treatment has been observed to result in near-complete removal of MC compounds (Bruchet et al. 1998) but the high dosage requirements generate significant cost barriers. Although a combination of coagulation, sand filtration, ozonation and chlorination were shown to remove up to 6.8 μg L⁻¹ of dissolved MCs (Jurczak et al. 2005), these and other more complex treatments, including advanced oxidation processes, are expensive and may lead to the production of toxic or undesirable byproducts (Srinivasan & Sorial 2011). Biological treatments, on the other hand, have shown considerable promise as a low-cost removal of cyanobacterial metabolites. More than a dozen bacterial species have been identified in MIB and geosmin biodegradation studies (Ho et al. 2007a) and MC-LR has been reported to undergo significant degradation at the soil-water interface of natural waters (Grützmacher et al. 2009) and via slow-sand or bank filtration (Grützmacher et al. 2006).

Bank filtration is a process of collecting water from wells or infiltration galleries that are recharged by a river/lake that flows through an alluvial valley in which the wells or infiltration galleries are installed. An infiltration gallery is a set of perforated pipes installed underneath the river/lake sediment. Taihu lake sediment mainly consists of silty clay (Sun & Huang 1993) and has a relatively low hydraulic conductivity. As a result, an infiltration gallery directly installed below the lake sediment may not have a sufficient water production capacity. However, the production capacity can be greatly enhanced if the hydraulic conductivity of the lake bed is increased, e.g., by mixing the lake bed sediment with sand. Therefore, the primary aim of this study was to evaluate the degradation of MIB, geosmin and MC-LR in sand filters containing a proportion of Taihu bed sediment by conducting bench-scale column experiments and transport modeling.

**METHODS**

**Column preparation**

Sediment was collected from the lake bed in Meiliang Bay (31°32′42″ N, 120°11′58″ E) using a grab sampler. The sediment, which has been previously characterized as silty clay with a median diameter of 0.012 mm (Sun & Huang 1993), was mixed with unwashed, medium to coarse (0.5–1 mm diameter) quartz-sand (Binjiang Water Treatment Plant, Changshu, China) in ratios (w/w) of 5/95 (E1) and 10/90 (E2). The sediment/sand mixtures were wet-packed into 30 cm long glass columns with an inner diameter of 5.0 cm. A control column (C1) was packed with 10/90 sediment/sand mixture that was autoclaved at 120 °C for 15 min. Once packed, the experimental and control columns were acclimated for a period of 4 weeks by pumping unfiltered lake water using a peristaltic pump (Masterflex, Cole-Parmer) at 0.57 mL min⁻¹ (corresponding to an average linear velocity of 1 m d⁻¹).

**Influent**

Influent was prepared by pumping 10 L of filtered (0.22 μm) lake water into two 15 L gas-tight Teflon bags (Beijing Safelab Technology Co.). When the bags were half-full, 300 μL of MIB/geosmin standard (Sigma Aldrich) and 400 μL of MC-LR standard (Express Bio-technology Co., Beijing) were injected into the inlet port and pumping was resumed until the total influent volume of each bag was 10 L, resulting in an estimated influent concentration of 15 μg L⁻¹ for each standard. It is noted that no headspace existed in the Teflon bags.

**Column experiments**

The influent bags were connected to the columns using Teflon tubing, and influent was injected through the columns using the peristaltic pump at a constant rate of 0.57 mL min⁻¹. To prevent volatile losses of dissolved compounds, effluent samples were collected by affixing a gas-tight 20 mL syringe (Hamilton Company, Reno, NV) to a three-way stop-cock on the outlet port on the top of the column. When the syringe was full, the contents were
quickly transferred to 20 mL glass vials, sealed, and stored at 4 °C. On the first day of pumping, samples were collected at approximately 0.5, 1, 1.5, 2 and 2.5 pore volumes (PV). Thereafter, samples were collected at 8 hour intervals (approximately 1 PV) until 20 PV had been exchanged. When the experiment (Expt A) was complete, the columns were re-acclimated for 1 week, fresh influent was prepared, and the experiment was repeated in full (Expt B). It has previously been suggested that some degradation of MC-LR occurs in the lake water itself (Chen et al. 2008), which may cause the influent MC-LR to degrade during the course of the experiment. To eliminate this potential degradation route, a third set of column experiments was conducted using distilled water in place of influent analysis.

Effluent analysis

MIB and geosmin concentrations in all samples were analyzed using solid-phase microextraction (SPME). After transferring 10 mL of effluent to a septum-capped vial containing a clean magnetic stir-bar and 4 g NaCl, the vial was immersed in a water bath at 65 °C and stirred constantly. The SPME fiber (Supelco #57348U) was introduced into the head space of the vial and equilibrated for 10 min. The fiber was then manually inserted into the inlet of an Agilent 6890 gas-chromatograph coupled with an Agilent 5973i mass spectrometer. An Agilent DB-5MS capillary column (30 m×0.25 mm, 0.25 μm) was used to separate the compounds. The initial oven temperature was 50 °C. After a 2 min hold, the oven temperature was ramped to 190 °C at 10 °C min⁻¹, to 280 °C at 15 °C min⁻¹, and held for 10 min. The concentration of MIB and geosmin was subsequently quantified with reference to the internal standards 2-isopropyl-3-methoxypyrazine (IPMP, Sigma Aldrich) and geosmin-D3 (MW=114).

MC-LR samples were analyzed by liquid chromatography tandem mass spectrometry (LC/MS/MS) with electron spray ionization (ESI) and multiple reaction monitoring (MRM). A Shimadzu Prominance UFLC with an Agilent Eclipse Plus C18 (1.8 μm×4.6 mm×50 mm) column was used for separation. The temperature of the column oven was kept at 40 °C. The mobile phase consisted of water (component A) and methanol (component B) buffered with 0.1% (v/v) formic acid and 4 mM ammonium formate. The following gradient elution program was used: 55% B for 0.5 min, linear increase from 55% B to 95% B from 0.5 to 6.0 min, 95% B from 6.0 to 6.5 min, and linear decrease from 95% B to 55% B from 6.6 to 7.6 min. The flow rate was 1.0 mL min⁻¹.

Mass spectrometry was performed with an ABI 4000 Q-trap mass spectrometer (Applied Biosystems, Carlsbad, CA, USA), with nitrogen as both the collision gas and nebulizing gas. The curtain gas, collision gas, and ion source gas 1 and gas 2 were set at 25, 5, 60 and 60 psi, respectively. The nebulizer current was set at 3 mA. The temperature of the interface heater was maintained at 500 °C. Identification of MC was made by four MRM transitions, and quantification was based on the most abundant transition and external standards.

Transport modeling

The breakthrough curves of MIB and geosmin were fitted to a one-dimensional transport model that incorporates reversible sorption and first-order decay of solutes in porous media. The numerical model HYDRUS-1D version 4.0 (Simunek et al. 1998) was used, with the following partial differential equation:

\[
\frac{\partial C}{\partial t} + \frac{\rho_b}{\theta} \frac{\partial S}{\partial t} = D \frac{\partial^2 C}{\partial x^2} - \nu \frac{\partial C}{\partial x} - \mu_L C - \frac{\rho_b}{\theta} \mu_S S
\]  \hspace{1cm} (1)

where \(C\) is the solute concentration in aqueous phase, \(S\) is the solute concentration on solid phase, \(t\) is the time, \(\rho_b\) is the sediment bulk density, \(\theta\) is the porosity, \(D\) is the hydrodynamic dispersion coefficient, \(\nu\) is the average linear velocity, \(x\) is travel distance (length of column), \(\mu_L\) is the aqueous-phase first-order decay constant, and \(\mu_s\) is the solid-phase first-order decay constant. A linear isotherm was used to describe possible sorption of the solutes by the sediment:

\[
S = k_{dC}
\]  \hspace{1cm} (2)

where \(k_{dC}\) is the partitioning constant. Coupling the linear sorption isotherm with Equation (1) yields:

\[
\left(1 + \frac{\rho_b}{\theta} k_{dC}\right) \frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} - \nu \frac{\partial C}{\partial x} - \mu_T C
\]  \hspace{1cm} (3)
where $\mu_T$ is the overall first-order degradation constant given by:

$$
\mu_T = \mu_L + \frac{\rho_b k_d}{\theta} \mu_S
$$

(4)

Once the sorption coefficient ($k_d$) and first-order degradation constant ($\mu_T$) were obtained from inverse models, a set of direct models were run to estimate performance of a 2 m thick filtration bed for the removal of MIB, geosmin and MC-LR. The filtration distance (the depth of the perforated pipes underneath the bed surface) of an infiltration gallery is typically more than 2 m.

**RESULTS AND DISCUSSION**

**Removal of MIB**

During reactive transport, sorption would only lead to retardation of the breakthrough of a solute and would not lower the steady-state breakthrough (plateau) concentration. Only degradation would result in steady-state $C/C_0$ of less than 1. MIB breakthrough was observed at approximately 1 PV in each column (Figure 1) and the shape of the breakthrough curves (i.e., no obvious retardation) is consistent with the low sorption coefficients derived from transport models (Table 1).

In contrast to the sterile column, where little to no MIB removal was observed (Figure 1, top left panel), the removal of MIB from columns containing 5 and 10% lake bed sediment (w/w) averaged 11 and 24%, respectively (Figure 1, lower left panels). Fitted first-order degradation rate constants were essentially zero for the control column (Table 1), and increased to 0.48 and 1.08 $d^{-1}$ for the 5/95 and 10/90 columns, respectively. MIB removal appeared proportional to the amount of sediment in the column. Since the only difference between experimental column E2 and the control column is sterilization, it is safe to state that the degradation is biotic. Our results demonstrated that only a 10% (w/w) addition of lake sediment to a sand column could support a healthy number of microbes to degrade MIB.

The degradation rate constants observed here were a full order of magnitude higher than those achieved in rapid filtration through biologically active sand columns (Ho et al. 2007a), even at the lower sediment ratio. Forward models show that degradation in a 2 m thick sand bed with 10%
lake sediment could remove as much as 84% of influent MIB (Figure 2). When the concentration of MIB in Taihu is less than 5 ng L\(^{-1}\), infiltration galleries incorporating lake sediment may require little or no additional treatment for MIB. When productivity is high, traditional treatment such as PAC or GAC may be required to bring the final concentration of MIB down to acceptable levels, but pretreatment using an infiltration gallery with a sediment mixture would significantly reduce dosage requirements and their associated costs.

### Removal of geosmin

The breakthrough of geosmin was slightly retarded with respect to the MIB breakthrough, indicating that geosmin was more strongly sorbed to the column material. Fitted \( k_d \) values for geosmin were much higher than those for MIB (Table 1), consistent with the visual inspection. In column E1 (5/95), removal is relatively low, averaging 8%, but the addition of an extra 5% sediment in column E2 (10/90) led to a significant increase in geosmin removal, reaching an average of 38.5% (Table 1). Again, fitted degradation rate constants for the control column were zero, whereas the rate constants increased to an average of 0.26 and 1.68 d\(^{-1}\) for the 5/95 (E1) and 10/90 (E2) columns, respectively (Table 1). Since the difference between experimental column (E2) and the control column (C1) is sterilization, the degradation is likely biotic. It is worth noting that sorption may enhance overall degradation if at least some degradation occurs on the solid phase (see Equation (4), where an increase in \( k_d \) would increase \( \mu_T \)). An increase in the amount of lake sediment would not only increase the number of microbes but also the number of sorption sites (i.e., \( k_d \) for columns E2 and C1, each with a 10% sediment ratio, was higher than that for E1, with only 5% sediment). Often microbial mediated degradation of contaminants in porous media occurs in the solid phase (Weissenfels et al. 1992). As such, increasing sorption would be beneficial to the overall contaminant removal, as seen in geosmin degradation in columns E1 and E2.

![Figure 2](https://iwaponline.com/ws/article-pdf/12/5/691/416947/691.pdf)

**Figure 2**  |  Forward model results for removal of MIB (solid line) and geosmin (dashed line) in a 2 m column of lake bed sediment and quartz sand (10/90).

<table>
<thead>
<tr>
<th>Column</th>
<th>Expt</th>
<th>Sorption coefficient ((k_p, L/kg))</th>
<th>Degradation constant ((\mu_T, d^{-1}))</th>
<th>Percent removal (%)</th>
<th>Sorption coefficient ((k_p, L/kg))</th>
<th>Degradation constant ((\mu_T, d^{-1}))</th>
<th>Percent removal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>A</td>
<td>0.05</td>
<td>0.000</td>
<td>0</td>
<td>0.4</td>
<td>0.000</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>0.08</td>
<td>0.12</td>
<td>3</td>
<td>0.5</td>
<td>0.000</td>
<td>0</td>
</tr>
<tr>
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<td>0.48</td>
<td>11</td>
<td>0.2</td>
<td>0.48</td>
<td>14</td>
</tr>
<tr>
<td>B</td>
<td></td>
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<td>0.48</td>
<td>11</td>
<td>0.01</td>
<td>0.048</td>
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<tr>
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<td>0.96</td>
<td>22</td>
<td>0.3</td>
<td>1.68</td>
<td>39</td>
</tr>
<tr>
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<td>1.2</td>
<td>26</td>
<td>0.7</td>
<td>4.68</td>
<td>38</td>
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</tbody>
</table>

**Table 1**  |  Percentage removal of MIB and geosmin and transport parameter values determined by inverse modeling of experimental breakthrough data

The fitted degradation rate constant \( (\mu_T) \) for experimental column E2 (Table 1), containing 10% lake bed sediment, was significantly higher than those reported for rapid sand filtration (Ho et al. 2007a). Thus, the addition of bed sediment appears to have the potential to degrade geosmin at high rates if the flow is relatively slow. The results of this experiment indicated, like previous studies, that dissolved geosmin was more readily removed from water than MIB. In batch experiments, these observations may be influenced by the higher vapor pressure and volatility of geosmin. Here though, care was taken to ensure no volatile losses occurred prior to sampling and analysis, so the higher rate of removal was a function of degradation alone. Forward models...
suggested that up to 96% of influent geosmin could be removed using a 2 m thick sand bed with 10% lake sediment (Figure 2). Even when productivity of Taihu is high the dissolved concentration of geosmin rarely exceeds 10 ng L⁻¹ (Chen et al. 2013). Therefore, an infiltration gallery coupled with additional volatile losses during traditional treatment may satisfactorily treat influent geosmin throughout the year, significantly reducing the cost associated with traditional treatment.

Removal of MC-LR

MC-LR has previously been observed to undergo rapid degradation in natural waters, which helps to maintain lower concentrations that are predicted by biological productivity (Chen et al. 2008). Although lake water used as influent was filtered at 0.22 μm, the apparent degradation of MC-LR in influent samples (measured at 0, 3, 6, 9, 12, 15 and 18 PV) indicated that some microbial activity was still present. A second attempt to quantify influent samples, 1 week after initial quantification, showed that all of the influent had since decayed beyond detectable levels, so the first set of column experiments was deemed inconclusive and repeated using an influent prepared with distilled water. In the control column (sterilized, 10/90), there is a brief breakthrough (C/C₀=0.94) of MC-LR after 1 PV has been exchanged, followed by a relatively linear decay (Figure 3). As the experiment proceeded, the effluent concentration gradually decreased to about 20% in the first experiment. By the time 8 PV had been exchanged in the replicate experiment, the concentration of MC-LR had dropped below detectable levels. Attempts to model the data using HYDRUS 1D were unsuccessful, but the lack of retardation in the initial breakthrough indicated that MC-LR was poorly sorbed to the column material, so degradation, either abiotic or biotic, accounted for the observed MC-LR losses. Since MC-LR is generally stable towards chemical degradation routes (Chen et al. 2008), biotic degradation is more likely. The columns had previously been used with lake water, which appeared to have been biologically active, so it is possible that small microbial communities were established in the sterilized column during the first set of experiments. Following an initial acclimation period (1–2 PV), microbial growth stimulated by MC-LR would account for the observed pattern of degradation as the experiment progressed. The results from the control column indicated that MC-LR would readily undergo biodegradation, even at low microbial concentrations. Analytical results from experimental columns E1 and E2, where no MC-LR breakthrough occurred, supported this conclusion. These and other studies (Holst et al. 2005; Ho et al. 2007b, 2010) indicate that complete removal of MC-LR is possible under a variety of conditions that promote biodegradation.

CONCLUSIONS

The objective of this study was to evaluate the biodegradation rate of MIB, geosmin and MC-LR in sand filters containing a proportion of Taihu bed sediment. Breakthrough curves for MIB and geosmin were well-fitted
using the transport model (Table 1) and showed that the addition of 10% lake bed sediment (w/w) to sand resulted in 24 and 38.5% removal of influent MIB and geosmin, respectively, at a flow rate of 1 m d$^{-1}$. Forward models estimated that extending the column length to 2 m increased removal of MIB to 84% and geosmin to 96%. MC-LR was completely degraded in columns with both 5 and 10% proportions of lake bed sediment in 30 cm columns, indicating that an infiltration gallery with a 2 m filtration distance would also achieve the desired removal rates. Future research will determine degradation products of MIB, geosmin and MC-LR, assess degradation rates at higher flow rates, and evaluate whether local sediments can degrade other known taste/odor compounds such as dimethyl trisulfide and related alkyl sulfide compounds, which have been attributed to the main septic smell observed during the 2007 bloom (Yang et al. 2008).

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