Biological filtration for the removal of algal metabolites from drinking water


*CRC for Water Quality and Treatment, Australian Water Quality Centre, PMB 3, Salisbury SA 5108, Australia (E-mail: lionel.ho@sawater.com.au)
**Georg-Simon-Ohm University of Applied Science, Wassertorstrasse 12, Nuremberg 90478, Germany
***Technical University of Berlin, Strasse des 17.Juni 135, Berlin D-10623, Germany

Abstract Biological sand filters were assessed for their ability to remove geosmin, 2-methylisoborneol (MIB) and microcystin-LR. Microcystin-LR was the most readily degradable metabolite with a maximum lag period of only 5 days before it was undetected in the filter effluent. Geosmin and MIB were difficult to degrade, with a period in excess of 75 days before greater than 95% removal was achieved. A microcystin-degrading gene was detected in the biofilm from one of the filters, confirming that the biofilm possessed the ability to degrade microcystin. A Sphingomonas sp. was identified as a potential geosmin degrader based on denaturing gradient gel electrophoresis (DGGE) analysis. DGGE analysis revealed a more complex bacterial community during the degradation of MIB, suggesting that more than one bacterium may be responsible for its degradation.

Keywords Biological filtration; degradation; geosmin; MIB; microcystin

Introduction

The prevalence of algal blooms in water sources is a major problem for water authorities as a large proportion of these blooms are able to produce metabolites which are recalcitrant to conventional water treatment processes. Of major concern to water authorities are geosmin, 2-methylisoborneol (MIB) and the microcystins toxins. Both geosmin and MIB impart earthy/musty tastes and odours (T&Os) in drinking water which often lead to customer complaints; while the microcystins are hepatotoxins which have also been implicated as carcinogens. Consequently, effective removal of these metabolites from drinking water is paramount.

Biological filtration systems for the removal of contaminants are becoming more attractive to water suppliers as they are generally of low technology, requiring little maintenance and infrastructure. Geosmin, MIB and the microcystins have been reported to be degraded by aquatic bacteria (Izaguirre et al., 1988; Egashira et al., 1992; Saadoun and El-Migdadi, 1998; Bourne et al., 1996, 2001; Grützmacher et al., 2002). However, only a few of these studies have reported degradation of these metabolites in biological sand filters.

Perhaps the most important aspect of the degradation of these metabolites is the identification of the degrading bacteria. To date, geosmin degradation has only been reported by genera of Gram-positive bacteria such as Bacillus (Narayan and Nunez, 1974), Arthrobacter and Rhodococcus (Saadoun and El-Migdadi, 1998). Genera of bacteria most often reported as degrading MIB include Bacillus (Ishida and Miyaji, 1992; Lauderdale et al., 2004) and Pseudomonas (Izaguirre et al., 1988; Egashira et al., 1992). Only a few strains of the genus Sphingomonas have been reported to degrade microcystin (Bourne et al., 2001; Park et al., 2001; Saito et al., 2003).
The diminutive population of bacteria responsible for the degradation of these metabolites suggests that specific genes are involved in the degradation processes. Bourne et al. (1996, 2001) identified a gene cluster in a *Sphingomonas* sp. which was responsible for the degradation of microcystin-LR. Recently, Saito et al. (2003) developed a polymerase chain reaction (PCR) for the detection of one of these genes, *mlrA*. Similarly, a mechanism for the degradation of MIB has been proposed involving the genes responsible for the degradation of camphor by *Pseudomonas putida* (Oikawa et al., 1995). However, the genes for the degradation of geosmin have not been ascertained.

The aim of this study was to determine if biological sand filters were capable of degrading geosmin, MIB and microcystin-LR. A further aim was to identify the bacteria, and possibly the genes, responsible for the degradation of the metabolites in the filters. Currently, there is no method to determine if a biological filter contains the bacteria capable of degrading these compounds. Therefore, it may be possible to develop molecular techniques to identify the genes responsible for the degradation of each metabolite. These techniques could be used as tools to monitor biological filters for their ability to degrade each metabolite, which would be of enormous value to the water industry, particularly since the occurrence of these metabolites is usually of a transient nature.

**Materials and methods**

**Chemicals and reagents**

Stock solutions of MIB and geosmin (Ultrafine Chemicals, UK) were prepared by dissolving in Milli-Q water (Millipore Pty Ltd, USA). Microcystin-LR (m-LR) was extracted from a bloom of *Microcystis aeruginosa*. All glassware, equipment and solutions were autoclaved (121°C for 15 minutes) when possible, otherwise sterilised through 0.2 μm cellulose nitrate membranes. Sample water was collected from the Myponga Reservoir, South Australia. Sand was obtained from the filter beds at the Morgan Water Treatment Plant (WTP).

**Biological filtration experiments**

Filter sand, collected from the Morgan WTP, was packed into three glass columns (length of 30 cm, ID of 2.5 cm) at a bed height of 15 cm. The columns were fed with Myponga Reservoir water (MRW) and spiked with either 20 μg/L of m-LR or 100 ng/L of MIB and geosmin. Samples were taken from the column influents and effluents at regular intervals for analysis of the respective metabolites. Experiments were conducted at room temperature (20 ± 2°C). At the completion of the column experiments, sand was removed from the columns and the biofilm detached by periodic vortexing for 15 minutes. The supernatant was collected and any carry over of sand was pelleted by slow speed centrifugation at 1,000 × g for 30 seconds. The supernatant was then washed twice by centrifugation at 1,000 × g for 15 minutes with resuspension of the pellet each time in sterile Bushnell-Haas (BH) minimal liquid medium (0.1% (w/v) NH₄NO₃, 0.1% (w/v) K₂HPO₄, 0.1% (w/v) KH₂PO₄, 0.01% (w/v) MgSO₄.7H₂O, 0.01% (w/v) FeCl₃.6H₂O and 0.001% (w/v) CaCl₂.2H₂O). The resulting supernatant, containing the biofilm bacteria, was used for the identification of possible metabolite degraders.

**Identification of microcystin degraders**

DNA was extracted from the supernatant using a DNA Preparation Kit (Bio-Rad, Australia), then used as a template for a PCR to detect the microcystin-degrading gene, *mlrA* (Saito et al., 2003). Amplifications were performed using a GeneAmp® 2400 PCR System (Perkin Elmer, Australia) using conditions described previously (Ho et al., 2005).
Identification of MIB and geosmin degraders

Attempts to enrich and isolate MIB and/or geosmin degrading bacteria were performed in liquid minimal medium supplemented with the T&O compounds as the sole carbon source. The number of active bacteria within the supernatant was enumerated using a FACSCalibur flow cytometer (Becton Dickinson, USA) following staining of the bacteria with the BacLight Kit (Molecular Probes Inc., USA), as reported previously (Hoefel et al., 2003). Approximately $1 \times 10^5$ active bacteria/mL were inoculated separately into 20 mL of BH minimal medium supplemented with approximately 20 mg/L of MIB or geosmin. To assess for any bacterial growth within the minimal medium alone an equivalent inoculum was prepared without the addition of MIB or geosmin. In addition, the biofilm inoculum was inactivated by autoclaving (121°C for 20 minutes) and this was inoculated separately into 20 mL of BH minimal liquid medium supplemented with concentrations of MIB or geosmin reported above. These cultures were used as a control to monitor the amount of MIB or geosmin loss due to factors other than biological degradation. Each culture was incubated at 22°C with continuous shaking. Samples were taken every 7 days for active bacterial enumeration and MIB and geosmin analyses. In addition, 0.5 mL aliquots of sample underwent DNA extraction by three equivalent cycles of boiling at 100°C for 5 minutes followed by freezing in liquid nitrogen for 1 minute. This DNA was used as a template for universal 16S rDNA-directed PCR with denaturing gradient gel electrophoresis (DGGE) analysis using a D-GENE™ Gel Electrophoresis System (Bio-Rad, USA) with DNA sequencing of target bands, as reported previously (Hoefel et al., 2005).

Analysis of the metabolites

Prior to high performance liquid chromatography (HPLC) analysis, m-LR was concentrated from sample waters by C18 solid phase extraction according to the methods described by Nicholson et al. (1994). Samples for MIB and geosmin analyses were pre-concentrated using a solid phase microextraction syringe fibre (Supelco, Australia) and analysed on a 5890 Series II Gas Chromatograph with 5971 Series Mass Selective Detector (Hewlett Packard, Australia). Full details of this analysis have been documented by Graham and Hayes (1998).

Results and discussion

Biofiltration of microcystin

Columns A and B were employed for the biofiltration of m-LR. Column A had been in operation for 12 months prior to the commencement of this study, during which it was fed with MRW with periodic spikings of m-LR. However, prior to the commencement of this study, column A had not been exposed to m-LR for 6 months. Column B contained sand which had been autoclaved prior to the commissioning of the experiments to inactivate the existing biofilm and to determine the extent of microcystin removal in the absence of an active biofilm.

Column A was fed with MRW spiked with m-LR for 39 days as shown in Figure 1. The initial empty bed contact time (EBCT) of the column was 30 minutes, although this was sequentially reduced down to a minimum of 7.5 minutes. During the initial stages of the experiment it was difficult to maintain a constant microcystin influent concentration due to degradation of m-LR in the influent water. This was resolved by cleaning the influent water reservoir and tubing at regular intervals to minimise any biological activity occurring prior to the sand filters.

No m-LR was detected in the effluent of column A at any time during the experiment, even at an EBCT of 7.5 minutes. In contrast, breakthrough of m-LR was observed in the
initial days of operation of column B. After 5 days, no m-LR was detected in the effluent of column B. This implies that the time required for a biofilm to establish and acclimatise to m-LR in this set of experiments was reasonably short, in contrast to other studies which have documented lag periods of up to 16 days prior to degradation of microcystin commencing (Miller and Hallowfield, 2001). Furthermore, the results strongly suggest that the removal of m-LR through the sand columns was through biological processes rather than any physical processes since the initial breakthrough of m-LR in column B was in the absence of an active biofilm.

Biofiltration of MIB and geosmin
Column C was employed for the biofiltration of geosmin and MIB at an initial EBCT of 15 minutes (Figure 2). Significant breakthrough of both T&O compounds was observed in the first 9 days after which the removal of both T&O compounds steadily increased. On day 79, the EBCT of the column was increased to 30 minutes, which corresponded to a further increase in the removal of geosmin and MIB (>95%). The removal trends of the T&O compounds was quite different to that of the microcystins where complete removal was observed within 5 days. This suggests that geosmin and MIB are more difficult to biologically degrade than the microcystins, which is similar to the oxidation trends of the metabolites. Geosmin and MIB are tertiary alcohols which may render their structures to be more resistant to breakdown than their toxic counterparts.

Detection of the microcystin degrading gene, mlrA
A previously published PCR (Saito et al., 2003) was employed to identify the microcystin degrading gene, mlrA in the biofilm of column A, which was shown to effectively degrade m-LR. The mlrA gene is important as it encodes an enzyme which has been shown to initiate the degradation of microcystin by cleaving the Adda-arginine peptide bond in m-LR, thereby opening up its cyclic structure (Bourne et al., 1996, 2001).
A homologous \textit{mlrA} gene was detected in the biofilm of column A (results not shown), confirming that the biofilm possessed the ability to degrade m-LR via this route. Full details of the \textit{mlrA} detection by the PCR test have been described previously by Ho et al. (2005). To date, no other studies have reported detection of the \textit{mlrA} gene in biological sand filters. The detection of the homologous \textit{mlrA} gene in this study indicates that this PCR can be used to assess biofilters for their microcystin degrading capability.

\textbf{Identification of geosmin and MIB degraders}

As the genes responsible for geosmin and MIB degradation have not been fully characterised, a different approach was employed to isolate and identify geosmin and MIB degraders within the biofilm of column C. This involved using liquid culture, with geosmin or MIB supplemented as the sole carbon source. For geosmin and MIB to be used as the primary growth substrate, mg/L concentrations were required, which are several orders of magnitude greater than the ng/L concentrations often encountered during T&O episodes in the environment. In those instances, bacterial growth is primarily supported by more assimilable NOM within the waters where geosmin or MIB are available as secondary substrates.

When geosmin was available as the sole carbon source for the biofilm inoculum, a 13 day period of minimal activity was observed. In the subsequent 22 day period, 15 mg/L of geosmin was assimilated resulting in a 1.31 log increase in active bacterial abundance (Figure 3a). DGGE analysis of the bacterial community within the liquid culture revealed a shift in community composition at the onset of geosmin degradation (day 13 onwards) where one band became predominant within the profile (Figure 3b). This band remained predominant throughout the remaining incubation period. DNA sequencing of the excised band revealed a sequence identical to a 169 bp fragment of the 16S rDNA gene from \textit{Sphingomonas} sp. It was hypothesised that this was the primary bacterium within the biofilm responsible for the removal of geosmin. Isolation of the bacterium on solid nutrient medium will be performed and growth of the isolated organism in minimal liquid medium supplemented with geosmin as the sole carbon source will further confirm its ability to degrade geosmin. This is the first report of geosmin degradation involving a species of the Gram-negative bacterium \textit{Sphingomonas}.

A 13 day period of minimal activity, similar to that for the removal of geosmin, was also observed when MIB was present as the sole carbon source for growth of bacteria from within the biofilm of column C. In the subsequent 22 day period, 17 mg/L of MIB was assimilated resulting in a 0.91 log increase in active bacterial abundance (Figure 4a).

![Figure 3](https://iwaponline.com/ws/article-pdf/6/2/153/418105/153.pdf)
DGGE analysis of the bacterial community within the liquid culture during the onset of MIB degradation (day 13 onwards) revealed a complex profile with no bands becoming discernibly predominant (Figure 4b). This was in contrast to the DGGE profile of the geosmin degrading culture (Figure 3b) indicating that the degradation of MIB may have involved many more bacteria in comparison to the degradation of geosmin. This may have been due to either a variety of biofilm associated bacteria each with the ability to degrade MIB in its entirety, or due to cross feeding between different organisms where a subset of bacteria from within the biofilm inoculum carried out the first stage of degradation, from which the products of the first stage fed a second subset of bacteria, which produced a third product, and so on. Further work will be conducted to verify this theory.

**Conclusions**

Geosmin, MIB and m-LR were readily degraded in biological sand filters. Microcystin-LR was by far the easiest metabolites to biologically degrade with a maximum lag period of only 5 days before the microcystins were effectively removed. In contrast, the T&O metabolites were difficult to degrade, with a period in excess of 75 days before greater than 95% removal was observed.

The detection of a homologous \textit{mlrA} gene in the biofilm of column A indicates that there is potential for PCR to be used as a tool to rapidly assess biological filters for their ability to degrade microcystin. Based on DGGE analyses, a \textit{Sphingomonas} sp. was implicated as a possible geosmin degrader, while for MIB, a range of unidentified bacteria were thought to be responsible for its degradation. Once the identity of these unknown bacteria can be ascertained and confirmed, it should be possible to investigate and identify the genes involved in the degradation processes, which could then result in a molecular tool similar to that of the PCR used to identify the \textit{mlrA} gene for microcystin degradation.

**References**


