Removal of cyanobacterial metabolites through wastewater treatment plant filters
Lionel Ho, Daniel Hoefel, Charlotte Grasset, Sebastien Palazot, Gayle Newcombe, Christopher P. Saint and Justin D. Brookes

ABSTRACT
Wastewaters have the potential to proliferate excessive numbers of cyanobacteria due to high nutrient levels. This could translate to the production of metabolites, such as the saxitoxins, geosmin and 2-methylisoborneol (MIB), which can impair the quality of wastewater destined for re-use. Biological sand filtration was assessed for its ability to remove these metabolites from a wastewater. Results indicated that the sand filter was incapable of effectively removing the saxitoxins and in some instances, the effluent of the sand filter displayed greater toxicity than the influent. Conversely, the sand filter was able to effectively remove geosmin and MIB, with removal attributed to biodegradation. Granular activated carbon was employed as an alternative filter medium to remove the saxitoxins. Results showed similar removals to previous drinking water studies, where efficient removals were initially observed, followed by a decrease in the removal; a consequence of the presence of competing organics which reduced adsorption of the saxitoxins.

INTRODUCTION
The prevalence of cyanobacteria in drinking water sources has received widespread attention due to these organisms possessing the ability to produce metabolites which can impair the quality of drinking water. In particular, cyanobacteria such as *Anabaena* have the ability to produce compounds such as geosmin and 2-methylisoborneol (MIB) which can impart tastes and odors (T&Os), causing numerous consumer complaints. However, and more importantly from a health perspective, *Anabaena* can also produce toxins, such as the saxitoxins, which can have a detrimental impact on human health. The saxitoxins, also known as paralytic shellfish poisons, are alkaloid neurotoxins which can damage nerve cells and cause death if consumed in sufficient quantity (Kao 1993). Currently, no guideline level exists for the saxitoxins, although the Australian Drinking Water Guidelines has issued a provisional health alert level of 3 μg/L as saxitoxin toxicity equivalents (STX-equivalents) (NHMRC 2004).

Over the years, significant research has been undertaken to ensure that these metabolites are well-removed during drinking water treatment; however, there has been little published with respect to their removal in wastewater treatment plants (WWTPs). Nutrient levels are considerably greater in wastewaters than drinking water sources, and with increasing ambient temperatures, there is greater risk of cyanobacterial blooms in wastewater stabilization lagoons which could potentially translate to greater levels of cyanobacterial metabolite production (Vasconcelos & Pereira 2001; Paerl & Huisman 2009).

The onset of climate change and drought, particularly in countries such as Australia and the USA has resulted in a need to seek alternative water sources. Recycled wastewater is one of these sources; consequently, there is a need to ensure that the quality of this water is of a standard that can be used appropriately. In many countries recycled wastewater is used for irrigation purposes and
as cyanobacterial toxins have been reported to accumulate in crops, in addition to adversely affecting the growth of crops (Codd et al. 1999; Pflugmacher et al. 1999), it is imperative that this water is free of these toxins to ensure that exposure to humans via crop-based foods is minimized.

In recent times, biological sand filtration has been documented to be effective in removing a range of cyanobacterial metabolites from drinking water sources (Ho et al. 2006, 2007; McDowall et al. 2009). However, minimal studies have assessed biological sand filtration in wastewaters for this purpose. It should not be assumed that the previous observations for drinking water can be directly translated to wastewaters due to the different chemical composition, matrix and microbial populations. If biological sand filtration has the ability to remove cyanobacterial metabolites during wastewater treatment, it may also be viable to employ this treatment for the removal of wastes generated from processes such as membrane filtration and in more recent times anionic exchange resin treatment. These processes generate highly concentrated organic streams that could also contain a high concentration of cyanobacterial metabolites (both in intracellular and extracellular forms). In such an environment the cyanobacterial cells have a high propensity to lyse, releasing large amounts of intracellular metabolites.

The aim of this study was to assess the effectiveness of biological sand filtration to remove cyanobacterial metabolites, primarily the saxitoxins, from a tertiary treated wastewater destined for re-use. To date, only one study has investigated the biological filtration of saxitoxins which was conducted using a drinking water source (Kayal et al. 2008). As a secondary aim, the biological sand filtration of geosmin and MIB was assessed as these compounds are often simultaneously produced with the saxitoxins. While it is acknowledged that the cyanobacterial T&Os are usually of greater relevance to drinking water, evidence suggests that high concentrations of geosmin and MIB could lyse cyanobacteria (Ikawa et al. 2001), potentially releasing high concentrations of intracellular saxitoxins into wastewaters. Moreover, geosmin and MIB were added as a comparator with the saxitoxins as the data will also provide useful information on how a range of organic compounds might be removed during biological filtration processes. A previous study has shown that geosmin could be used as a surrogate for saxitoxin during powdered activated carbon treatment (Ho et al. 2009).

EXPERIMENTAL PROCEDURES

Materials and reagents

Tertiary treated effluent (TTE) water was obtained from the Bolivar WWTP in Adelaide, South Australia. This water was sampled prior to the sand filters at the tertiary dissolved air flotation filtration (DAFF) plant and used for laboratory-scale sand and granular activated carbon (GAC) column experiments and batch degradation experiments. The treatment processes upstream of TTE water include preliminary grit removal, primary sedimentation, secondary activated sludge treatment and 20 d detention in waste stabilization lagoons prior to the DAFF plant. In addition, activated sludge treated effluent (ASTE) water was used for laboratory-scale sand column experiments for short periods of time. Characteristics of these waters are presented in Table 1. At the time of sampling, both waters did not contain background levels of saxitoxins, geosmin or MIB.

Five saxitoxin variants were used in this study: C1, C2, GTX2, GTX3 and STX. These were extracted from a bloom of Anabaena circinalis that occurred in Myponga Reservoir, South Australia. The isolation and purification procedures employed have been documented previously (Ho et al. 2010). The resultant toxin stock solution had a profile characteristic of Australian strains of A. circinalis where the toxin variants C1 and C2 were predominant with smaller quantities of GTX2, GTX3 and STX variants (Velzeboer et al. 2000). Saxitoxin standards used in this study were purchased from the National Research Council of Canada.

Geosmin and MIB were obtained from a commercial supplier (Ultrafine Chemicals, UK) and dissolved in ultrapure water (Millipore Pty Ltd, USA) to prepare stock solutions. Aliquots from these stock solutions were then dosed into the water at the desired concentrations.

Table 1 | Comparison of water quality parameters for tertiary treated effluent (TTE) water and activated sludge treated effluent (ASTE) water

<table>
<thead>
<tr>
<th>Parameters</th>
<th>TTE water</th>
<th>ASTE water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrate (mg L⁻¹)</td>
<td>7.1 ± 2.6</td>
<td>18.7 ± 1.8</td>
</tr>
<tr>
<td>Ammonia (mg L⁻¹)</td>
<td>2.3 ± 1.9</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>Phosphorus (mg L⁻¹)</td>
<td>2.4 ± 1.6</td>
<td>5.3 ± 0.7</td>
</tr>
<tr>
<td>DOC (mg L⁻¹)</td>
<td>8.4 ± 0.8</td>
<td>11.3 ± 0.7</td>
</tr>
<tr>
<td>COD (mg L⁻¹)</td>
<td>107 ± 41</td>
<td>143 ± 30</td>
</tr>
<tr>
<td>BOD (mg L⁻¹)</td>
<td>4 ± 5</td>
<td>1 ± 2</td>
</tr>
</tbody>
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**Laboratory column experiments**

Biological sand filtration experiments were conducted using laboratory-scale glass columns. Sand samples, collected from the Bolivar DAFF plant, were packed into the columns (length 30 cm, I.D. of 2.5 cm) at a bed height of 15 cm. The sand media had an effective size of 0.95 mm with a uniformity coefficient of 1.3. The columns were fed with TTE water spiked with saxitoxins, geosmin and MIB. The columns were operated at empty bed contact times (EBCTs) of 15 or 30 min (corresponding to filtration rates of 0.6 and 0.3 m/h, respectively) and were backwashed once a week with deionized water for 5 min with 10% bed expansion. Previous studies have shown that backwashing with deionized water had no effect on the biofilm and its ability to remove cyanobacterial metabolites (Ho et al. 2006, 2007). Experiments were conducted at room temperature (22–25°C).

A laboratory GAC filtration experiment was conducted using the same procedure as that of the laboratory sand filtration experiment, with the exception of the media where virgin GAC was used instead of sand. In addition, only the GAC filtration of saxitoxins was evaluated. The GAC employed (F400) was a coal-based steam activated carbon which was obtained from a commercial supplier (Calgon, USA). The GAC media had an effective size of between 0.55 and 0.75 mm, a uniformity coefficient of 1.9 and iodine number of 1,000 mg/g.

**Batch degradation experiments**

Batch experiments were conducted to assess the degradation of the cyanobacterial metabolites by indigenous microorganisms within the TTE water. In these experiments, 2 L Schott Pyrex bottles, containing metabolite-spiked TTE water, were adopted as reactors. In addition, parallel control reactors containing sterilized (autoclaved at 121°C for 15 min) TTE water were prepared to assess losses of the contaminants due to physical processes. The reactors were incubated aerobically at room temperature (22–25°C) with constant stirring. Samples were aseptically taken from each reactor at regular time intervals for analysis of the metabolites.

**Analyses**

Saxitoxin analyses were conducted using a high performance liquid chromatography (HPLC) system comprising a 600 pump controller and 717 plus autosampler with post-column derivatization and detection using a 2,475 multi λ fluorescence detector (Waters Pty Ltd, Australia). Full details are given in Rositano et al. (1998). Concentrations of the saxitoxins were determined by calibration of the peak areas with that of certified reference standards (Institute of Marine Biosciences, National Research Council, Canada). Conversion factors (Oshima 1998) were used to express the toxicity of the sum of the variants as STX-equivalents due to the differing toxicities and concentrations of the individual saxitoxin variants.

Where HPLC analysis was not available (e.g. laboratory GAC and batch degradation experiments), a commercially available enzyme linked immunosorbent assay (ELISA) was employed for the analysis of saxitoxins according to the manufacturer’s instructions (Abraxis, USA).

Samples for geosmin and MIB analyses were preconcentrated using a solid phase microextraction syringe fiber (Supelco, Australia) and analyzed using a gas chromatography-mass spectrometry system (Agilent Technologies, Australia) against quantified labelled internal standards (Ultrafine Chemicals, UK). Full details of this method have been documented by Graham & Hayes (1998).

**RESULTS AND DISCUSSION**

**Biological sand filtration experiments**

The saxitoxin concentration (expressed as STX-equivalents) in the influent and effluent of the laboratory sand filter is shown in Figure 1. Minimal removal of the saxitoxins
(average of 6%) was observed within the first 285 d of operation of the filter. The EBCT was doubled to 30 min on day 285 in an attempt to initiate removals by allowing the saxitoxins to reside for a longer period of time within the sand filter. On day 327, the sand filter influent water was switched to ASTE water to seed the filter with a greater diversity (and possibly numbers) of organisms to facilitate biodegradation, as ASTE is sampled upstream of TTE water at the WWTP. Both methods proved to be futile as only a minor increase in removal of the saxitoxins was observed up to day 404 (average increase of 4%). The inefficient saxitoxin removal through the sand filter is consistent with Kayal et al. (2008) where negligible removal of saxitoxins was evident through drinking water sand filters. However, Kayal et al. (2008) observed an increase in toxicity through anthracite filters which was attributed to biotransformation of the less toxic C1 and C2 variants to the more toxic GTX2 and GTX3 variants.

In this study there were instances where the effluent STX-equivalent concentrations were slightly higher than the influent STX-equivalent concentrations (see Figure 1). It was presumed that this was due to some form of biotransformation of the individual saxitoxin variants, and evidence of this is shown in Figure 2. For example, between days 99 and 109 there was a gradual decrease in the concentration of C1 in the effluent of the sand filter which was coupled with a concomitant increase in the concentration of GTX2. Concentrations of the individual variants remained at the same consistent ratio in the influent (results not shown). These results suggest that the organisms located within the biofilm of the sand filter possessed the ability to mediate these biotransformations, albeit for only short periods of time, possibly due to the dynamic nature of biofilms (Sutherland 2001). Previous studies have shown that C1 can be biotransformed into GTX2 (Bricelj et al. 1991; Cembella et al. 1993). Those studies documented these conversions occurring within the tissues of marine organisms. In addition, Kotaki et al. (1985) demonstrated that marine bacteria, isolated from coral crabs and marine snails, were capable of biotransforming saxitoxins.

The biotransformations of the C-toxins to GTX variants have important implications for the water industry as these conversions resulted in the toxicity of the effluent water increasing. Although biological filtration has been shown to be effective for the removal of other cyanobacterial metabolites, there may be an issue with using this option for the treatment of the saxitoxins, as the increases in toxicity of the filtered water have the potential to compromise human health.

Figures 3(a) and (b) show the concentrations of geosmin and MIB, respectively, through the laboratory sand filter. The sand filter was able to efficiently remove both geosmin
and MIB with only two instances where slight breakthrough of the compounds was observed. An abatement in geosmin and MIB spiking was practiced between days 25 and 55 to simulate transient conditions which are generally observed for these cyanobacterial metabolites (Scharf et al. 2014). Upon recommencement of the spiking after 30 d, geosmin and MIB was still being efficiently removed. These efficient removals were also observed in a drinking water study by Ho et al. (2010), highlighting the effectiveness of biological sand filtration for the removal of these T&O compounds. However, other studies have shown geosmin and MIB to not be as well removed through sand filters (Elhadi et al. 2006; McDowall et al. 2009), suggesting that the removal of these compounds may be site specific and highly dependent upon the indigenous organisms present in the influent waters and biofilms of the sand filters. Previous studies have isolated organisms responsible for the degradation of geosmin and MIB from drinking water sources and within the biofilms of sand filters, confirming their biodegradability in aquatic environments (Eaton & Sandusky 2009; Hoefel et al. 2009). Efforts were made to isolate the organisms responsible for the degradation of geosmin and MIB in this study, but these were unsuccessful.

The effective removals of the T&O compounds by the sand filter indicate that they cannot be used as a surrogate to evaluate saxitoxin removal through such filters. While geosmin has previously been shown to be a potential surrogate for saxitoxin adsorption using activated carbon (Ho et al. 2009), the same cannot be said during biological filtration, possibly due to the different organisms required for the degradation of the specific metabolite.

**Batch degradation experiments**

Batch experiments were performed with the saxitoxins to determine if the TTE water contained organisms with the ability to degrade them over time (Figure 4(a)). There was a decrease of ~40% in the saxitoxin concentration in the TTE water over the course of the experiments; however, this decrease was also observed in the sterilized control reactor, suggesting that the losses of saxitoxins in TTE water was due to non-biological processes. Previous studies have documented that saxitoxin concentrations can decrease abiotically due to changes in pH and temperature (Indrasena & Gill 2000). However, in this study the pH (7.5 ± 0.1) and temperature (23.2 ± 0.6 °C) were consistent throughout the batch experiment indicating that these were not the contributing factors for the reduction of saxitoxin concentrations. It is also unlikely that photodegradation played a role in the reduction of the toxins as saxitoxins have a very weak UV absorbance.

Efficient removals of geosmin and MIB by the sand filter when fed with TTE water was attributed to biological action, in particular, due to the organisms within the TTE water. To confirm this, batch experiments were conducted for both T&O compounds in TTE water (Figures 4(b) and (c)). Geosmin was degraded down to 9 ng/L from an initial concentration of 40 ng/L; a reduction of approximately 75% (Figure 4(b)). Negligible reduction of geosmin was evident in the sterilized control reactor, confirming that biodegradation was the removal mechanism.

The degradation of geosmin was determined to follow a pseudo-first-order mechanism which is in agreement with previous studies in drinking water (Ho et al. 2007; Hoefel et al. 2009). However, the geosmin degradation rate constant was calculated to be 0.073 d⁻¹ (equivalent to 0.003 h⁻¹,
$R^2 = 0.87$, which is an order of magnitude lower than those reported by Ho et al. (2007) and Hoefel et al. (2009). The lower rate constant in this study may be attributed to a number of factors, including the water matrix, where the greater level of more assimilable organics and nutrients in wastewater could possibly result in slower geosmin uptake by the degrading organisms. Furthermore, the studies by Ho et al. (2007) and Hoefel et al. (2009) utilized either an enriched biofilm (which was extracted from a sand filter shown to degrade geosmin) or an isolated geosmin-degrading bacterium as the inoculum in sterilized reservoir water; consequently, the geosmin-degrading organisms in those waters had no competition with any indigenous organisms present in the waters.

Conversely, no reduction in MIB concentration was evident during the batch experiment (Figure 4(c)). It is possible that the organisms responsible for MIB degradation may be inhibited when in a planktonic state compared with a biofilm-associated state. Studies have demonstrated that certain bacterial populations exhibit different patterns of gene expression when in a biofilm compared with a planktonic state; furthermore, biofilm-mediated bioremediation has been documented to be more efficient than planktonic bioremediation as it is believed that cells within a biofilm are more protected within the matrix and hence can more easily adapt to changing conditions (WST-Decho 2000; Whiteley et al. 2001).

**GAC filtration experiment**

The results of the sand filter and batch experiments suggest that there were no organisms capable of degrading saxitoxins within the biofilm of the sand filter or the TTE water and that sand filtration (and possibly any form of biological treatment) would be an ineffective process for saxitoxin removal at the Bolivar WWTP. Consequently, a laboratory GAC filter was evaluated for its ability to remove the saxitoxins. GAC filters have been identified as an alternative option as a polishing step in wastewater treatment for the removal of a range of water quality parameters (Lin & Lai 2000; Chaudhary et al. 2005). It was decided not to investigate the GAC filtration of geosmin and MIB as sand filtration had already been shown to be effective for their removal through biological action.

To date, only a few studies have evaluated GAC filtration for the removal of saxitoxins, and of those, none have been conducted in wastewater (Newcombe 2002; Ho et al. 2010). Those studies showed GAC filtration to be an effective process for saxitoxin removal from drinking water sources.

The results from this study showed GAC to be effectively removing saxitoxins on commissioning with >99% removal.
(Figure 5). After approximately 1 month the GAC was still able to remove approximately 96% of the saxitoxins. After 6 months the removal of saxitoxins decreased to approximately 75%. The reduction in adsorption capacity of the saxitoxins can be attributed to the presence of organics which reduce adsorption of the compound of interest through competition for adsorption sites and/or through pore blockage mechanisms (Newcombe 2002; Li et al. 2005). Moreover, these results strongly suggest that removal of the saxitoxins was predominantly through adsorption mechanisms rather than biological processes.

Interestingly, these results were similar to those of Newcombe (2002) who conducted laboratory GAC column trials operating at an EBCT of 15 min. Newcombe (2002) showed that their GAC column was able to remove 100% of saxitoxins on commissioning, approximately 95% after 1 month operation, and approximately 70% after 5 months operation. These results suggest that the differences between drinking water sources and wastewater matrices had little impact on the GAC adsorption of the saxitoxins.

**SUMMARY AND CONCLUSIONS**

This study has shown that sand filtration was ineffective in removing saxitoxins. In some instances, the effluent STX-equivalent concentration was greater than the influent concentration and evidence suggested this to be due to some biotransformation of the saxitoxins variants, in particular, C1 being converted to GTX2. This has significant implications in using sand filtration to treat saxitoxins in wastewater as there is the potential that the effluent may be more toxic than the influent. In contrast, sand filtration has the potential to be an effective treatment option for geosmin and MIB, with removals attributed to biodegradation.

GAC filtration was able to effectively remove the saxitoxins initially; however, after 6 months removals decreased to 75% due to competition with organics present in the TTE water. Interestingly, the results mirrored that of a previous study conducted in a drinking water source, potentially implying that the GAC adsorption of saxitoxins is similar regardless of the water matrix.

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