

Optimising water treatment practices for the removal of *Anabaena circinalis* and its associated metabolites, geosmin and saxitoxins

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ABSTRACT

The cyanobacterium *Anabaena circinalis* has the ability to co-produce geosmin and saxitoxins, compounds which can compromise the quality of drinking water. This study provides pertinent information in optimising water treatment practices for the removal of geosmin and saxitoxins. In particular, it demonstrates that pre-oxidation using potassium permanganate could be applied at the head of water treatment plants without releasing intracellular geosmin and saxitoxins from *A. circinalis*. Furthermore, powdered activated carbon (PAC) was shown to be an effective treatment barrier for the removal of extracellular (dissolved) geosmin and saxitoxins, with similar adsorption trends of both compounds. The relative removal of the saxitoxins compared with geosmin was determined to be 0.84 ± 0.27 , which implies that saxitoxin removal with PAC can be estimated to be approximately 60 to 100% of the removal of geosmin under equivalent conditions. Chlorine was shown to be effective for the oxidation of the saxitoxins with CT values of approximately 30 mg min l^{-1} required for greater than 90% destruction of the saxitoxins.

Key words | *Anabaena circinalis*, chlorination, geosmin, potassium permanganate, powdered activated carbon (PAC), saxitoxin

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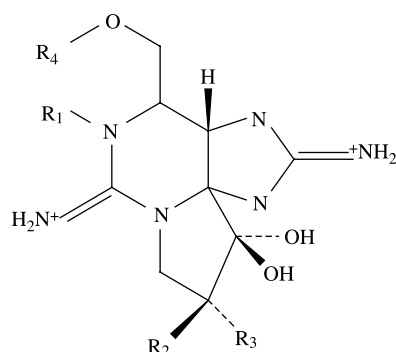
INTRODUCTION

Cyanobacteria (blue-green algae) are problematic for water authorities as they have the ability to produce metabolites which can compromise the quality of potable water, in particular, metabolites which can cause aesthetic issues (e.g. compounds which impart tastes and odours) and those which can severely affect human health (e.g. cyanobacterial toxins). In Australia, one of the predominant and most troublesome species of cyanobacteria is *Anabaena circinalis*. This cyanobacterium plagues drinking water sources and has the ability to simultaneously produce geosmin and saxitoxins.

Geosmin is a tertiary alcohol which imparts an earthy/musty odour in drinking water; its presence in drinking water at levels greater than 10 ng l^{-1} often results in

numerous complaints for water authorities. Although the presence of geosmin in treated water does not necessarily imply that the water is unsafe for consumption, it does indicate that there may be a problem with the treatment process.

The saxitoxins are a group of alkaloid neurotoxins which block nerve cell sodium channels and can cause death if consumed in sufficient quantity (Kao 1993). These toxins exist as variants, of which there are approximately 27, the most common being the C-toxins, gonyautoxins (GTX) and saxitoxin (STX). Of these three classes, the doubly sulphated C-toxins are the least toxic, followed by the more potent singly sulphated GTX variants, and finally STX which is non-sulphated and the most toxic.



	R ₁	R ₂	R ₃	Net charge	Relative toxicity
R ₄ = CONH ₂ (carbamate toxins)					
STX	H	H	H	+2	1.000
GTX2	H	H	OSO ₃ ⁻	+1	0.359
GTX3	H	OSO ₃ ⁻	H	+1	0.638
R ₄ = CONHSO ₃ ⁻ (n-sulfocarbamoyl (sulfamate) toxins)					
C1	H	H	OSO ₃ ⁻	0	0.006
C2	H	OSO ₃ ⁻	H	0	0.096

Figure 1 | Structural variations and characteristics of the saxitoxin class of cyanotoxins.

Figure 1 shows the general structure of the saxitoxins including some characteristics of the individual variants. Although no guideline value exists for the saxitoxins, a provisional health alert value of $3.0 \mu\text{g l}^{-1}$ (as saxitoxins toxicity equivalents, or STX-eq) has been suggested by Fitzgerald *et al.* (1999) for the Australian Drinking Water Guidelines (NHMRC 2004). The term STX-eq is commonly used as this reveals the relative toxicity of the water studied by knowing the ratio of toxicity of each saxitoxin variant in relation to the most toxic variant STX (see Figure 1 for relative toxicity of each variant). Expressing the combined toxicity in this form is more relevant from a health perspective since each individual variant varies significantly in its concentration and toxicity level.

Substantial research has been conducted in optimising coagulation processes in conventional water treatment for the removal of cyanobacterial (and algal) cells (see review by Mouchet & Bonnelye 1998); in particular, the addition of oxidants such as chlorine, ozone and potassium permanganate at the head of water treatment plants (WTPs) prior to coagulation has been shown to increase the removal of algae (Montiel & Welté 1988; Plummer & Edzwald 2002; Chen & Yeh 2005, 2006). These oxidants alter the surface characteristics and charge of algae thus improving their removal during coagulation and flocculation. Although coagulants typically used in water treatment have minimal

effect on the cell integrity of algae (Velzeboer *et al.* 1995; Chow *et al.* 1998, 1999), oxidants such as chlorine have been shown to cause damage to algal cells (Lam *et al.* 1995; Peterson *et al.* 1995; Petrusevski *et al.* 1996; Pietsch *et al.* 2002; Plummer & Edzwald 2002; Tung *et al.* 2004; Jurczak *et al.* 2005). This is a concern as damage to the cells may result in the release of intracellular metabolites, including cyanotoxins, which are recalcitrant to conventional water treatment processes (Keijola *et al.* 1988; Himberg *et al.* 1989). Other concerns regarding the addition of these oxidants include their ability to produce carcinogenic disinfection by-products (e.g. chlorine and ozone), and their ability to cause colour problems in the water (e.g. potassium permanganate).

In Australia, the addition of potassium permanganate at the head of WTPs is generally practised to oxidise soluble manganese, thereby minimising the impact of particulate manganese downstream in distribution systems. However, studies have demonstrated that potassium permanganate can lyse algal cells (Petrusevski *et al.* 1996; Chen & Yeh 2005, 2006) with the dose of potassium permanganate resulting in cell damage shown to be species-dependent. Consequently, there exists a dilemma in applying potassium permanganate to achieve conflicting water quality goals. To date, minimal studies have assessed the effect of potassium permanganate on cyanobacterial species; in particular, no studies have evaluated the impact of potassium permanganate, at doses relevant to WTPs, on the release of intracellular geosmin and saxitoxins from *A. circinalis*.

In addition to intracellular geosmin and saxitoxins, there exists a portion of these metabolites which is extracellular, and it is this portion that is not removed by coagulation processes during conventional water treatment. In many countries, one of the primary barriers for the removal of extracellular (or dissolved) cyanobacterial metabolites during water treatment is powdered activated carbon (PAC) adsorption. Many studies have evaluated the PAC adsorption of geosmin (Lalezary-Craig *et al.* 1988; Graham *et al.* 2000; Cook *et al.* 2001; Newcombe & Cook 2002); however, little has been published with respect to the PAC adsorption of the saxitoxins. Moreover, no studies have published work comparing the removal trends of both metabolites. This would be of an enormous value to the

global water industry as both metabolites can be produced simultaneously by species of cyanobacteria, including *A. circinalis* in Australia. More importantly, this information would assist plant operators to make informed decisions on the types and doses of PAC required to effectively remove both metabolites.

Another water treatment barrier that can be applied to remove extracellular cyanobacterial metabolites is oxidation by chlorine. Although chlorination is largely ineffective for geosmin oxidation (Lalezary *et al.* 1986; Anselme *et al.* 1988; Glaze *et al.* 1990), it can be highly effective for the oxidation of cyanobacterial toxins, such as cylindrospermopsin and the microcystins (Acero *et al.* 2005; Ho *et al.* 2006, 2008; Xagorarakis *et al.* 2006; Rodriguez *et al.* 2007). However, studies relating the chlorination of saxitoxins are sparse, and those that have been conducted have shown that saxitoxins could be effectively oxidised by chlorine provided the pH was above 8 (Nicholson *et al.* 2003; Senogles-Derham *et al.* 2003). Furthermore, these studies were unable to relate the oxidation of saxitoxins under a practical water treatment situation, in particular, determine the chlorine exposure (CT) values required for effective saxitoxin oxidation.

The aims of this study were to: (1) assess the impact of potassium permanganate, at realistic WTP doses and conditions, on the release of intracellular geosmin and saxitoxins from *A. circinalis*; (2) evaluate two different PACs in two different waters for the removal of geosmin and the saxitoxins—a pertinent objective was then to relate the removal of geosmin to the removal of the saxitoxins by PAC; (3) evaluate the effectiveness of chlorine for the oxidation of saxitoxins and determine the CT values required for confident oxidation of the saxitoxins under conditions expected in a WTP.

With the increasing frequency of cyanobacterial detection in global water supplies, coupled with the changing climate the world is facing, it is imperative that water authorities employ and optimise successful treatment strategies for the mitigation of cyanobacteria and their metabolites. The Australian Drinking Water Guidelines, and more recently the Water Safety Plans developed by the World Health Organisation, stipulate that it is important to utilise multi-barrier treatment options to ensure these contaminants do not reach the customer tap. Results from

this study will provide new insights into optimising multiple water treatment practices for the removal of *A. circinalis* and its associated metabolites.

EXPERIMENTAL METHODS

Materials and reagents

A strain of *A. circinalis* was isolated from the Myponga reservoir, South Australia, in December 2007. This strain was capable of co-producing geosmin and saxitoxins, with a large proportion of these metabolites determined to be intracellular.

For experiments utilising extracellular geosmin and saxitoxins, a combination of materials and procedures was employed. Geosmin was purchased from a commercial supplier (Ultrafine Chemicals, UK) and dissolved in Milli-Q water (Millipore Pty Ltd, USA) to prepare a geosmin stock solution (typical concentration of $\sim 100 \text{ mg l}^{-1}$). Aliquots from this stock solution were then dosed into waters at the desired concentrations. A saxitoxin spiking solution was extracted and purified from the bloom material of the aforementioned strain of *A. circinalis*. This solution had a toxic 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).

Sample waters were collected from the Myponga and Happy Valley reservoirs in South Australia. In addition, treated waters were sampled from the Myponga and Murray Bridge WTPs in South Australia. The treated waters were collected after the coagulation, sedimentation and filtration steps, but before final disinfection, at the respective WTPs. Characteristics of all waters are presented in Table 1. For PAC experiments, water samples were sterilised (autoclaved at 121°C for 15 min) and filtered through pre-rinsed $0.45 \mu\text{m}$ cellulose nitrate membrane filters (Schleicher and Schuell, Germany) to negate any biological effects.

Analyses

Samples for geosmin analyses were pre-concentrated using a solid phase microextraction syringe fibre (Supelco, Australia) and analysed on a 5890 Series II Gas Chromatograph

Table 1 | Characteristics of sample waters and powdered activated carbons (PACs) employed in this study

Water	UV absorbance at 254 nm (cm ⁻¹)	DOC (mg l ⁻¹)	SUVA (l mg ⁻¹ m ⁻¹)	pH	
Myponga reservoir	0.412	11.8	3.5	7.7	
Happy Valley reservoir	0.308	8.2	3.8	7.9	
Myponga treated	0.091	5.1	1.8	7.3	
Murray Bridge treated	0.051	2.7	1.9	7.5	
PAC	Particle size (µm)	BET* surface area (m ² g ⁻¹)	BET average pore width (Å)	Total pore volume (cm ⁻³ g)	Iodine number
PAC-A	20	978	26	0.65	1099
PAC-P	20	926	29	0.67	958

*BET is the conventional and widely used term and method to measure surface area. BET stands for Brunauer, Emmett, and Teller, the three scientists who optimised the theory for measuring surface area using the Langmuir method. In addition, the BET method can also be used to determine other information such as gas uptake, micropore volume, and pore size distribution via adsorption and desorption isotherms (see Brunauer *et al.* 1938).

with 5971 Series Mass Selective Detector (Hewlett Packard, Australia) against quantified labelled internal standards (Ultrafine Chemicals, UK). Full details of this method have been documented by Graham & Hayes (1998).

Saxitoxin analyses were conducted using a high performance liquid chromatographic (HPLC) system comprising a 600 pump controller and 717plus autosampler (Waters Pty Ltd, Australia) with post-column derivatisation and detection using a 2475 multi λ fluorescence detector (Waters Pty Ltd, Australia). The procedures employed were modified from the method of Oshima (1995). 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 1995) were used to express the toxicity of the sum of the variants as STX-eq owing to the differing toxicities and concentrations of the individual saxitoxin variants.

Dissolved organic carbon (DOC) measurements were made on an 820 Total Organic Carbon Analyser (Sievers Instruments Inc., USA). UV absorbance (at 254 nm) measurements were carried out on a UV-1201 UV/VIS Spectrophotometer (Shimadzu Corporation, Japan). Specific UV absorbance (SUVA) was calculated using the equation: $SUVA = 100 \times (\text{UV absorbance at } 254 \text{ nm} / \text{DOC})$. Prior to DOC and UV analyses, samples were passed through pre-rinsed 0.45 µm cellulose nitrate filters (Schleicher and Schuell, Germany). The pH of the waters was measured on a

PHI 50 pH meter (Beckman Instruments, USA) which was calibrated with pH 4, 7 and 10 standard buffers (BDH, Australia).

Pre-oxidation experiments using potassium permanganate

A stock solution of potassium permanganate was prepared by dissolving 1 g of potassium permanganate in 1 l of Milli-Q water in a 1 l volumetric flask. For decay experiments in Myponga reservoir water, potassium permanganate was added from the stock solution to obtain the desired doses and residual concentrations monitored at various time intervals up to 60 min. Potassium permanganate concentrations were determined using the DPD-FAS titration method described in *Standard Methods* (1998). Experiments were conducted in pre-cleaned 250 ml glass amber bottles at room temperature (20 ± 2°C).

For pre-oxidation experiments, the isolated geosmin- and saxitoxin-producing strain of *A. circinalis* was spiked into Myponga reservoir water at cell densities of 2,000 and 50,000 cells ml⁻¹. A pre-determined potassium permanganate concentration was dosed into the cell-spiked waters and aliquot samples quenched at various time intervals with sodium thiosulphate and subsequently analysed for geosmin and saxitoxin concentrations. These samples were divided in two sub-samples: the first sample was immediately filtered through a GFC filter (Whatman, UK) to remove cellular material, then analysed for extracellular

geosmin and saxitoxins. The remaining sample was subjected to two cycles of microwave lysis (each of 45 s at 900 W using a Model N858E benchtop microwave (NEC, Australia)) before GFC filtration to analyse for total geosmin and saxitoxins (both the intracellular and extracellular components).

PAC adsorption experiments

Two commercially available PACs were employed in this study, PAC-P, a wood-based steam activated carbon, and PAC-A, a coal-based steam activated carbon. The PACs were dried in an oven at 105°C for 24 h, then cooled and stored in a desiccator prior to use. Table 1 lists some characteristics of the PACs tested.

An FMS6V (SEM, Australia) variable speed, six paddle gang stirrer with 7.6 cm diameter flat paddle impellers and B-Ker² gator jars (Phipps and Bird, Australia) containing 2 l of geosmin- or saxitoxin-spiked water samples were employed for the adsorption experiments. Two PAC doses were evaluated: 10 and 30 mg l⁻¹. Rapid mixing was conducted at a speed of 220 rpm ($G = 480 \text{ s}^{-1}$) throughout the experiment to ensure that PAC remained suspended in solution. Samples were taken at 0, 5, 15 and 70 min and immediately filtered through pre-rinsed 0.45 µm cellulose nitrate filters (Schleicher and Schuell, Germany) then stored at 4°C prior to geosmin or saxitoxin analyses. In addition, any losses of the metabolites other than PAC adsorption were controlled for in jar test experiments performed in the absence of PAC. Experiments were conducted at room temperature ($20 \pm 2^\circ\text{C}$).

Chlorination experiments

A chlorine stock solution of 3–5 g l⁻¹ was prepared by bubbling gaseous chlorine in Milli-Q water for a predetermined time, then stored at 4°C for at least 16 h prior to use. All chlorination experiments were performed in pre-cleaned 1 l glass amber bottles at room temperature ($20 \pm 2^\circ\text{C}$). For chlorine decay experiments, waters (at natural pH) were spiked with saxitoxins, then dosed with known concentrations of chlorine. Samples (100 ml) were taken at various time intervals for free chlorine residual

determination using the DPD-FAS titration method described in *Standard Methods* (1998).

For saxitoxin decay experiments, waters (at natural pH) were spiked with saxitoxins prior to chlorination. Chlorine was added from the chlorine stock solution to obtain the desired doses. Samples were quenched at various time intervals with sodium thiosulphate. In addition, waters were adjusted to pH 8 using a 0.1 M phosphate buffer, and chlorination experiments conducted as above to ascertain the effect of pH on the chlorination of saxitoxins. All samples were immediately filtered through pre-rinsed 0.45 µm cellulose nitrate filters (Schleicher and Schuell, Germany) then stored at 4°C prior to saxitoxin analyses.

RESULTS AND DISCUSSION

Pre-oxidation using potassium permanganate

Potassium permanganate is generally dosed at the Myponga WTP at a stoichiometric ratio of 2:1 for soluble manganese. The mean concentration of soluble manganese in Myponga reservoir water over 2006–2007 was $0.018 \pm 0.021 \text{ mg l}^{-1}$ ($n = 49$, max = 0.077 mg l^{-1} , min = 0.001 mg l^{-1}). Consequently, this would have resulted in a potassium permanganate dose of $\sim 0.04 \text{ mg l}^{-1}$ based on the ratio of 2:1. However, this ratio does not take into consideration the consumption of potassium permanganate by factors such as the natural organic material (NOM) in the water. A 1 h decay experiment was undertaken in Myponga reservoir water using potassium permanganate doses of 0.25 and 0.50 mg l⁻¹ (Figure 2). The 0.25 mg l⁻¹ dose resulted in a residual of 0.06 mg l⁻¹ being detected after 1 h. This would have been sufficient to account for the mean concentration of soluble manganese; however, there were instances during 2006–2007 where this dose would not have been sufficient. Therefore, it was decided to employ a potassium permanganate dose of 0.50 mg l⁻¹ for subsequent experiments as this dose resulted in a residual of $\sim 0.16 \text{ mg l}^{-1}$ being detected after 1 h which would have been sufficient to account for the maximum soluble manganese level over the previous 2 years.

For the pre-oxidation experiments potassium permanganate was dosed at 0.50 mg l⁻¹ into Myponga reservoir water containing *A. circinalis* at cell densities of 2,000 and

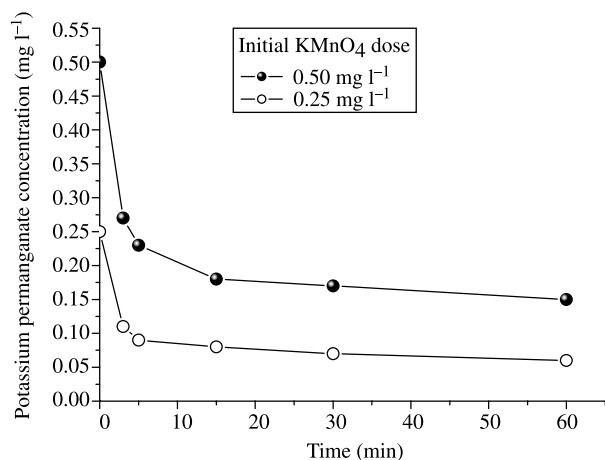


Figure 2 | Decay of potassium permanganate in Myponga Reservoir water using doses of 0.25 and 0.50 mg l⁻¹.

50,000 cells ml⁻¹. Samples were collected at 0, 5, 15, 30 and 60 min for analysis of total and extracellular geosmin concentrations. Total and extracellular saxitoxin concentrations were only evaluated for the 0, 15 and 60 min samples. Results are displayed in Figures 3 and 4 for geosmin and saxitoxins, respectively. The results for the saxitoxins were reported as STX-eq.

Geosmin and saxitoxins concentrations (total and extracellular) remained stable throughout the experiments, indicating that dosing a relatively high concentration (0.50 mg l⁻¹) of potassium permanganate had negligible effect on the *A. circinalis* cells, as determined by the absence of intracellular geosmin and saxitoxins release. The lack of oxidation of geosmin and saxitoxins by potassium permanganate is consistent with findings in the literature (Lalezary *et al.* 1986; Glaze *et al.* 1990; Rositano *et al.* 1998). This is attributed to the low oxidising potential of potassium permanganate, compared with oxidants such as chlorine and ozone, which themselves have been documented in some instances to be ineffective for the oxidation of geosmin and saxitoxins (Ho *et al.* 2002; Newcombe & Nicholson 2004).

These pre-oxidation experiments indicate that potassium permanganate can be dosed at the head of WTPs to achieve conflicting water quality goals; that is, not only to precipitate soluble manganese upstream of filtration, but also to enhance coagulation, without compromising the integrity of *A. circinalis* cells, based on the lack of intracellular release of geosmin and saxitoxins.

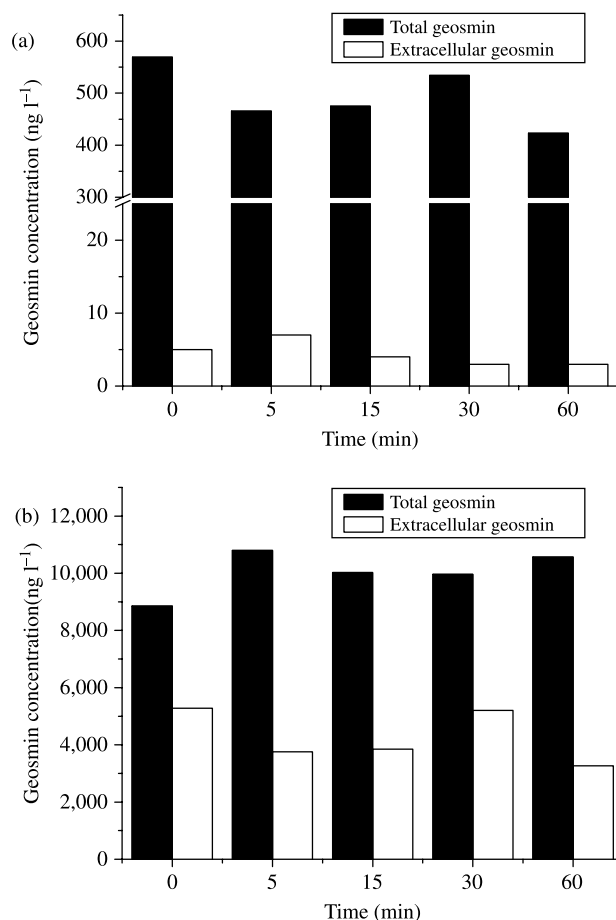


Figure 3 | Total and extracellular geosmin concentrations after treatment with potassium permanganate (0.5 mg l⁻¹) using *Anabaena circinalis* at numbers of: (a) 2,000 cells ml⁻¹; and (b) 50,000 cells ml⁻¹.

Therefore, it is possible that a large proportion of intracellular geosmin and saxitoxins can be removed through coagulation processes.

PAC adsorption of geosmin

PAC-A (coal-based) and PAC-P (wood-based) were evaluated for their capacity to adsorb geosmin from two drinking water sources, Myponga and Happy Valley reservoirs. The initial geosmin concentrations in the waters were between 70 and 80 ng l⁻¹. Figure 5 shows results from the adsorption experiments. No geosmin losses were evident in a control jar test in the absence of PAC. As expected, geosmin removal increased with increasing PAC dose and contact times in both waters. PAC-A was the

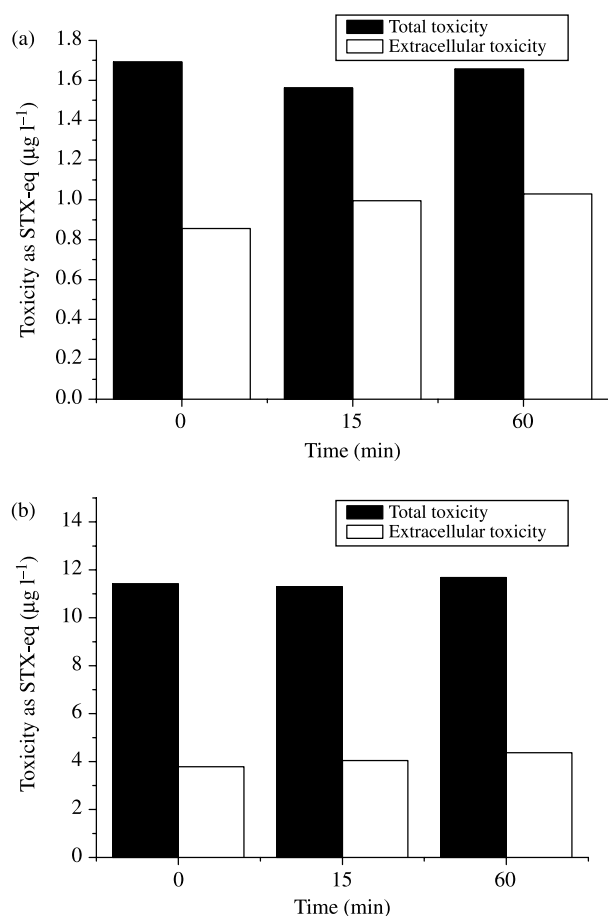


Figure 4 | Total and extracellular saxitoxins concentrations, expressed as STX-eq, after treatment with potassium permanganate (0.5 mg l^{-1}) using *Anabaena circinalis* at numbers of: (a) 2,000 cells ml^{-1} ; and (b) 50,000 cells ml^{-1} .

superior carbon for geosmin removal in both waters where a PAC-A dose of 10 mg l^{-1} achieved similar levels of geosmin removal to a PAC-P dose of 30 mg l^{-1} .

The difference in adsorption of geosmin by the two PACs may be related to the characteristics of the carbons. Studies have shown inferior adsorption of a similar cyanobacterial metabolite, 2-methylisoborneol, using wood-based carbons when compared with coal-based carbons (Chen *et al.* 1997; Newcombe *et al.* 1997; Pendleton *et al.* 1997). While both PACs in this study were described by their suppliers as microporous and mesoporous, it is generally observed that coal-based carbons contain a greater volume of micropores than their wood-based counterparts, which are favourable for the adsorption of smaller molecular weight compounds, such as geosmin (molecular weight of 182 g mol^{-1}) (Newcombe *et al.* 1997). Although both carbons displayed

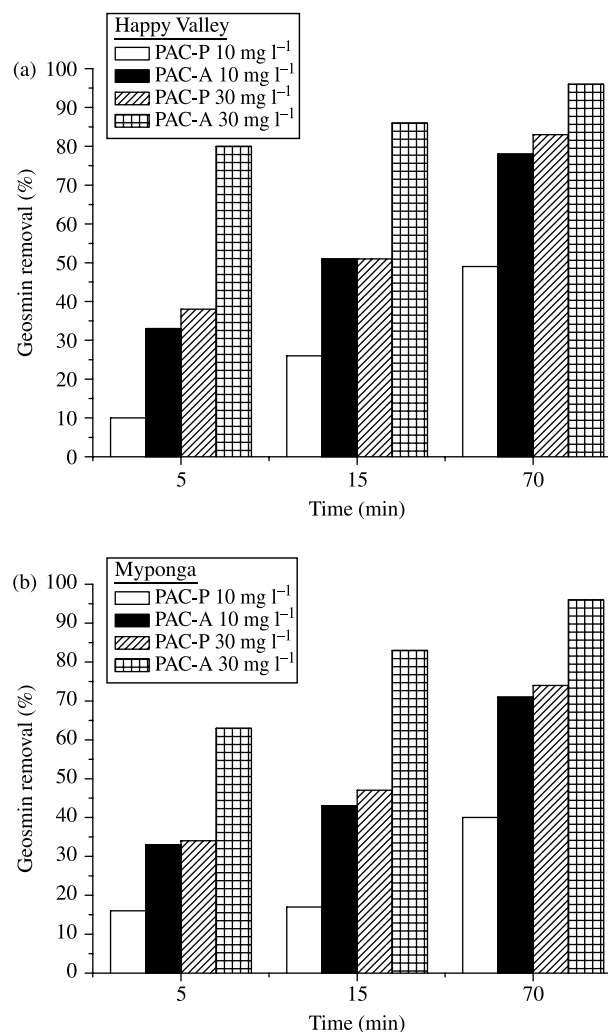


Figure 5 | Comparison of geosmin removal using two PACs at doses of 10 and 30 mg l^{-1} in: (a) Happy Valley Reservoir water; and (b) Myponga Reservoir water.

similar total pore volume values, PAC-A had a slightly smaller average pore size than PAC-P suggesting it may contain a greater number of micropores, which is consistent with the above contention. The slightly higher surface area and iodine number values of PAC-A provide further evidence for its greater geosmin adsorption capacity than PAC-P. In addition, Pendleton *et al.* (1997) determined that wood-based carbons contain a high oxygen content which produces a more hydrophilic surface resulting in lower adsorption energies and consequently lower adsorption for target contaminants.

While Myponga and Happy Valley reservoir waters were different in terms of their water quality characteristics

(see Table 1), only slight differences were observed in the adsorption of geosmin, with slightly greater geosmin removals apparent in Happy Valley reservoir water. Cook *et al.* (2001) also showed that waters of different quality had minimal impact on the adsorption of geosmin. NOM characteristics, such as DOC concentration and UV absorbance, have been shown to influence the adsorption of contaminants (Newcombe *et al.* 1997; Cook *et al.* 2001). However, the effect of NOM on the adsorption of geosmin in this study appears to be only minor suggesting that the carbons used had a wide range of pores which would offset the influence of NOM (Pelekani & Snoeyink 1999; Ebie *et al.* 2001; Newcombe *et al.* 2002; Li *et al.* 2003).

PAC adsorption of saxitoxins

The adsorption experiments for the saxitoxins were conducted in an identical fashion to the geosmin adsorption experiments. The degree of PAC adsorption was examined in terms of STX-eq removal. Figure 6 compares the adsorption of STX-eq using two PACs in Myponga and Happy Valley reservoir waters. No losses of the saxitoxins were evident in a control jar test experiment in the absence of PAC. Greater STX-eq removals were evident with increasing carbon dose and contact times in both waters. In addition, PAC-A was shown to be the more effective carbon for the removal of the saxitoxins, similar to the findings with the geosmin experiments. This again can be attributed to the different characteristics of the PACs, in particular, the greater surface area and more microporous nature of PAC-A. Cook *et al.* (2000) also showed varying levels of saxitoxin removals using a range of PACs and attributed the differences to the size of the saxitoxin molecules (molecular weights ranged from 256 to 491 g mol⁻¹) and the pore volume distributions of the PACs tested.

In the Happy Valley experiments, the initial STX-eq concentrations ranged between 3.7 and 4.1 µg l⁻¹, while in the Myponga experiments, initial concentrations ranged between 7.5 and 9.9 µg l⁻¹. These values are a realistic representation of levels that have been detected in these raw water sources. Using the provisional health alert value of 3.0 µg l⁻¹ (as STX-eq), a PAC-A dose of 10 mg l⁻¹ with a contact time of 15 min would be required to achieve removals to below this value in Happy Valley reservoir

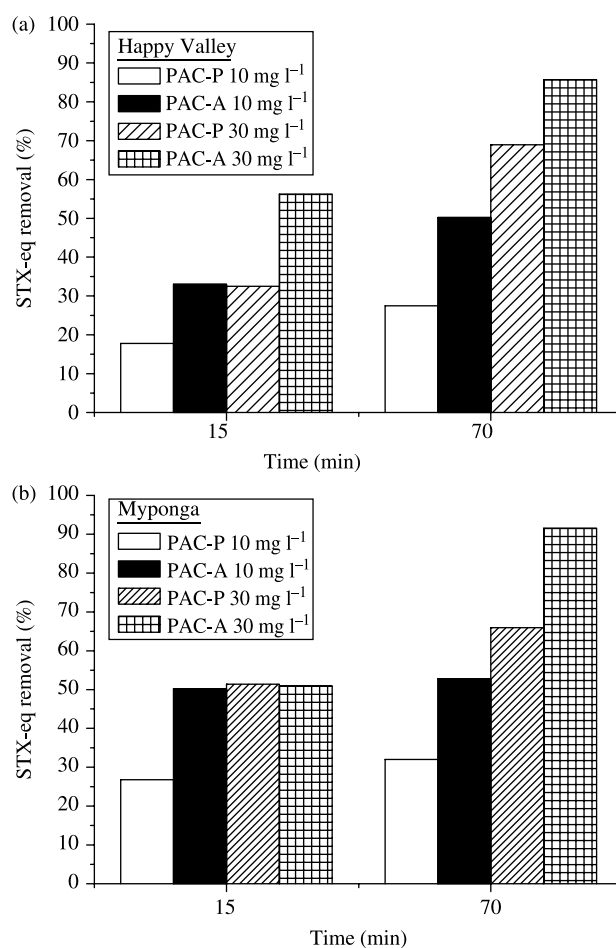


Figure 6 | Comparison of saxitoxin toxicity equivalent (STX-eq) removal using two PACs at doses of 10 and 30 mg l⁻¹ in: (a) Happy Valley Reservoir water; and (b) Myponga Reservoir water.

water, while a PAC-P dose of 30 mg l⁻¹ with a contact time of 70 min would be required. In Myponga reservoir water, a dose of 30 mg l⁻¹ with a contact time of 70 min would be required for both PACs to achieve the same level of removal. These PAC doses and contact times can be achieved in most WTPs, highlighting the effectiveness of PAC for the removal of saxitoxins.

The effect of water quality on the adsorption of the saxitoxins was minor with negligible difference observed between the removals in Happy Valley and Myponga reservoir waters. Similarly, only slight differences were observed in the adsorption of geosmin in both waters. These results suggest that lower competitive effects were evident and that this may be due to the wide range of pores of the carbons.

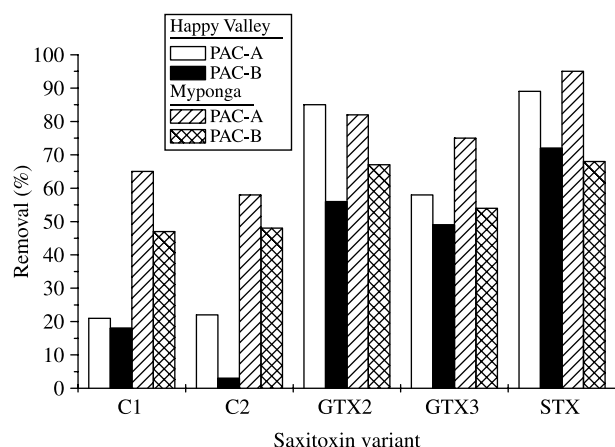


Figure 7 | Percentage removal of the individual saxitoxin variants by the two PACs in Happy Valley and Myponga waters (PAC dose of 30 mg l^{-1} , contact time of 70 min).

Differences were observed in the adsorption of the individual saxitoxin variants where the ease of adsorption of the variants by both PACs followed the trend: $\text{STX} > \text{GTX2} > \text{GTX3} > \text{C1} \approx \text{C2}$ (Figure 7). Cook *et al.* (2000) also reported similar adsorption trends using different PACs and attributed this to the size of the variants, with greater

removal with the lower molecular size of the variants. The authors also dismissed the notion that the charge of the variants (STX (+2), GTX (+1) and C-toxins (0)) may have influenced adsorption, with favourable adsorption using a carbon with a high positive surface charge. Interestingly, the adsorption trend also matches that of the toxicity; that is, the most toxic variant STX is also the most easily removed by PAC, while the C-toxins are the most difficult to remove.

Comparing the adsorption of geosmin with the saxitoxins

To date, no studies have attempted to relate the PAC adsorption of geosmin with that of the saxitoxins. This, in part, is due to the lack of studies investigating the PAC adsorption of the saxitoxins. Figure 8 is a graphical representation of the percentage removals of STX-eq and geosmin at 15 and 70 min for Happy Valley reservoir water. Under equivalent conditions, the removal trends for both compounds were similar in Happy Valley reservoir water using the two PACs. Similar observations were apparent for

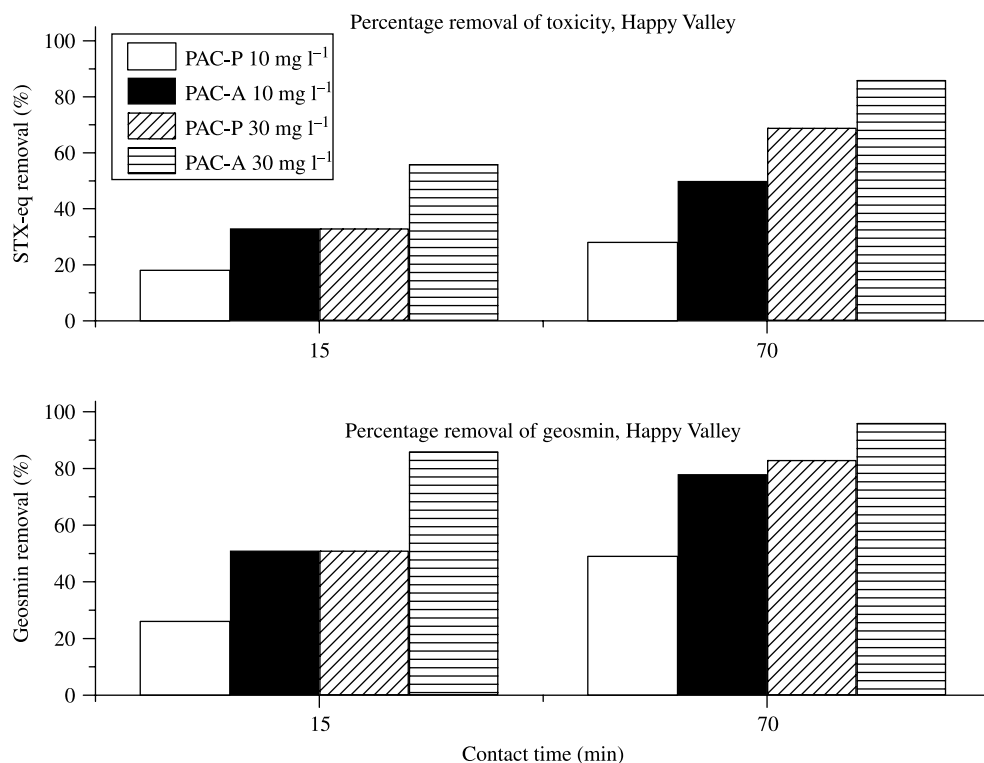


Figure 8 | Percentage removals of geosmin and saxitoxin toxicity equivalent (STX-eq) in Happy Valley water using two PACs at doses of 10 and 30 mg l^{-1} .

Myponga reservoir water (results not shown). The relative removal of STX-eq compared with geosmin in both waters using both PACs was calculated to be 0.84 ± 0.27 ($n = 16$). Based on this calculation, it is estimated that saxitoxin toxicity removal with PAC can be approximately 60 to 100% of the removal of geosmin under equivalent conditions. This implies that geosmin removal by PAC may be used as a surrogate to estimate saxitoxin toxicity removal, which can be beneficial as saxitoxin analyses are both expensive and labour intensive compared with geosmin analyses.

This result is of enormous value to the water industry as both compounds can be simultaneously produced by cyanobacteria, such as *A. circinalis*. For example, a recent study has shown that an *A. circinalis* density of 20,000 cells ml⁻¹ could potentially translate to toxin levels of 3.0 µg l⁻¹ (STX-eq) and geosmin levels of up to 900 ng l⁻¹ in Australian drinking water sources (Brookes & Burch 2007). As it is not uncommon to see *A. circinalis* reach levels in excess of 1,000,000 cells ml⁻¹ in Australia, this would result in significantly high concentrations of both compounds. Consequently, if plant operators are able to optimise PAC dosing to effectively remove geosmin, then it may be assumed that saxitoxin toxicity would also be effectively removed under equivalent conditions.

Chlorination of saxitoxins

Figure 9 shows the decay of chlorine (at initial chlorine doses of 1, 2 and 3 mg l⁻¹) in saxitoxin-spiked ($\sim 4 \mu\text{g l}^{-1}$ STX-eq) Murray Bridge and Myponga treated waters at the natural pH of the waters (ambient pH) and pH 8. The pH of the waters remained stable throughout the experiments. At both pH values, the two waters exhibited very similar chlorine decay curves at the lower chlorine doses of 1 and 2 mg l⁻¹. However, at the chlorine dose of 3 mg l⁻¹ a large difference was apparent between the waters, with more rapid decay in Myponga treated water. This can be attributed to the higher DOC, UV absorbance and SUVA values of Myponga treated water compared with Murray Bridge treated water. These characteristics have been shown to influence the reaction of chlorine, as NOM containing a higher proportion of conjugated and substituted aromatic moieties (and consequently light-adsorbing

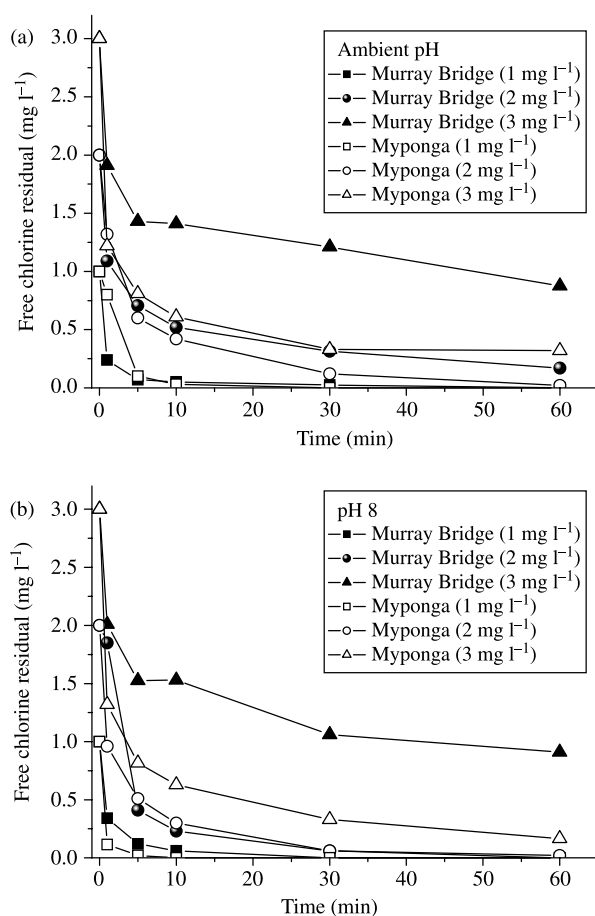


Figure 9 | Chlorine decay as a function of time in Murray Bridge and Myponga treated waters at: (a) ambient pH; and (b) pH 8.

chromophores) is more susceptible to chlorine attack (Reckhow *et al.* 1990; Korshin *et al.* 1997).

The chlorination of the saxitoxins ($\sim 4 \mu\text{g l}^{-1}$ STX-eq) in Murray Bridge and Myponga treated waters at ambient pH and pH 8 is shown in Figure 10. Efficient oxidation of the saxitoxins by chlorine was observed in all waters with CT values of approximately 30 mg min l⁻¹ required for greater than 90% oxidation of the saxitoxins, in terms of STX-eq. The ease of oxidation of the variants followed the trend STX > GTX3 ~ C2 > GTX2 ~ C1 (data not shown) which is consistent with the trends documented by Nicholson *et al.* (2003). The oxidation of the saxitoxins did not appear to be pH dependent, with minimal difference observed between pH 8 and ambient pH (Murray Bridge pH 7.5, Myponga pH 7.3). This is in contrast to the study by Nicholson *et al.* (2003) who observed greater saxitoxin oxidation with higher pH (pH range of 4–9). The authors

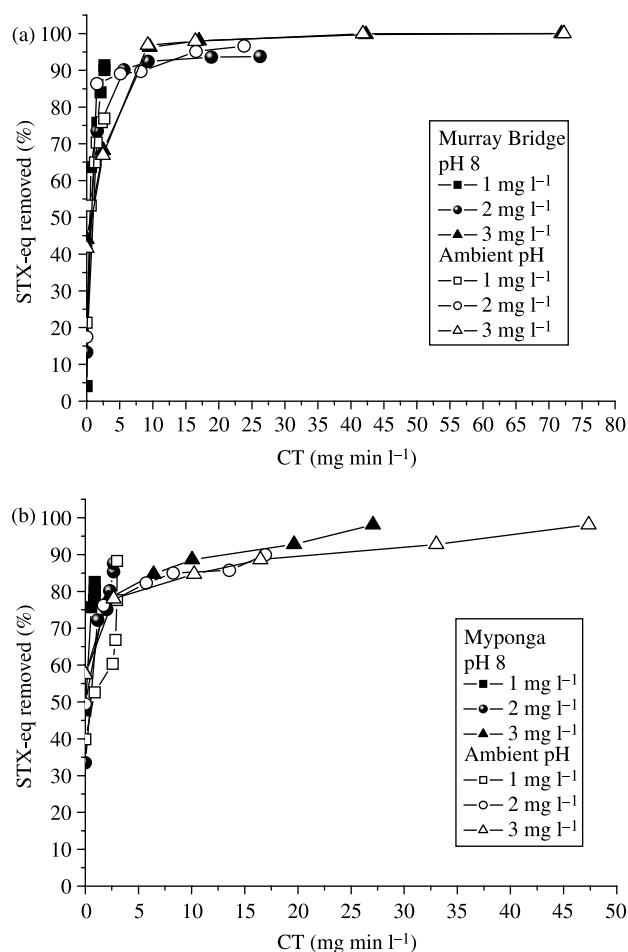


Figure 10 | Saxitoxin oxidation, expressed as STX-eq, as a function of CT using chlorine doses of 1, 2 and 3 mg l⁻¹ in: (a) Murray Bridge treated water; and (b) Myponga treated water.

attributed this to the toxin molecule being present in an unprotonated form at higher pH, and therefore more susceptible to oxidation, even though chlorine is known to be a weaker oxidant under these conditions. The results in this current study indicate that the saxitoxins have greater susceptibility to chlorine in natural waters than has previously been reported. This is fortuitous from a water treatment standpoint since the pH of most waters prior to final disinfection is between 7 and 8. Furthermore, the CT value required for effective saxitoxin oxidation corresponds to conditions which can be achieved in most WTPs.

More rapid oxidation of the saxitoxins was observed in Murray Bridge treated water when compared with Myponga treated water. In Murray Bridge treated water, a CT value of between 10 and 20 mg min l⁻¹ resulted in 95%

removal of STX-eq, while the same CT range in Myponga treated water only resulted in 80% removal. This can be explained by the higher NOM concentration in Myponga treated water compared with Murray Bridge treated water which results in greater competitive reactions between chlorine and NOM and chlorine and the saxitoxins.

CONCLUSIONS

Geosmin and saxitoxins can be simultaneously produced by *A. circinalis*. Their effective removal from drinking water is of paramount importance as they not only have the ability to cause aesthetic problems, but can also compromise human health. This study provided pertinent information in optimising multiple water treatment practices for the effective removal of geosmin and saxitoxins. In particular, results from this study indicate that:

1. The practice of pre-oxidation with potassium permanganate, at a concentration to treat for high soluble manganese levels, did not compromise the cell integrity of *A. circinalis*, as determined by the lack of intracellular geosmin and saxitoxin release. This also has the potential to optimise the coagulation of *A. circinalis* cells which could remove a large proportion of these metabolites.
2. PAC was an effective treatment barrier for the efficient removal of geosmin and saxitoxins. The adsorption trends of geosmin and the saxitoxins (based on STX-eq) were similar. The relative removal of STX-eq compared with geosmin was calculated to be 0.84 ± 0.27 , which implies that saxitoxin removal with PAC can be estimated to be approximately 60 to 100% of the removal of geosmin under the same conditions. Furthermore, the type of PAC was shown to be important for the adsorption of these metabolites with a coal-based carbon shown to be superior to a wood-based carbon in two different reservoir waters.
3. The saxitoxins have greater susceptibility to chlorine in natural waters than has previously been reported, with similar oxidation at ambient pH (7.3–7.5) and pH 8. Efficient oxidation of the saxitoxins by chlorine was observed in two treated waters with CT values of approximately 30 mg min l⁻¹ required for greater than 90% oxidation of the saxitoxins, in terms of STX-eq.

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