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Treatment options for the saxitoxin class of cyanotoxins

G. Newcombe and B. Nicholson

Australian Water Quality Centre, PMB 3, Salisbury, SA 5108, Australia
(E-mail: *gayle.newcombe@sawater.sa.gov.au*)

Abstract The saxitoxin class of algal toxins (cyanotoxins) are neurotoxins produced in Australia by the blue-green alga (cyanobacterium) *Anabaena circinalis*. A range of water treatment processes was investigated for the removal of these compounds. Ozonation of the toxins at moderate doses was found to be ineffective. Chlorination was found to be effective at high pH; therefore where pH adjustment is possible in the treatment process, chlorination could be considered as an important treatment option. Activated carbon, both in the granular and powdered form, was effective for reducing the toxicity of a mixture of the toxins as the most toxic of the saxitoxins were also the most readily removed by adsorption.

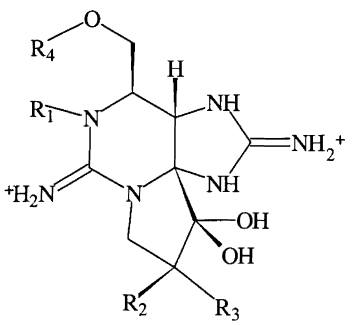
Keywords Algal toxins; blue-green algae; drinking water treatment; saxitoxins

Introduction

Saxitoxins are a class of neurotoxins produced by cyanobacteria (blue-green algae) and dinoflagellates that are found in both freshwater and marine environments. Relatively recently identified as the toxins produced by *Anabaena circinalis* in freshwater in Australia (Humpage *et al*., 1993), saxitoxins have also been identified in the United States, South America and Europe in blooms of several cyanobacterial species. The extent to which saxitoxins are found in drinking water worldwide can only be determined through a more systematic analysis of blooms of cyanobacteria, as recently demonstrated in Denmark, where saxitoxins were found to be widespread (Kaas and Henriksen, 2000).

The saxitoxins are a group of carbamate alkaloids ranging in molecular weight from 256 to 491. They can have a net charge of +2, +1 or 0, and display a wide range of toxicities. Structures and toxicities of the saxitoxins found in Australian *A. circinalis* are shown in Figure 1.

A range of saxitoxins is produced during a cyanobacterial bloom. In Australia the less toxic *C* toxins predominate, whereas a predominance of the more toxic GTX toxins and saxitoxins was recently reported in Denmark (Kaas and Henriksen, 2000). Neurotoxins affect the nervous system; they operate by a range of mechanisms, but can all cause death rapidly if consumed in sufficient quantity (Falconer, 1994). Viable drinking water treatment options are therefore of vital importance to water authorities sourcing water from reservoirs and rivers affected by saxitoxins.



	R1	R2	R3	Net Charge	Rel. toxicity
R4=$CONH_2$ (carbamate toxins)					
STX	H	H	H	+2	1
GTX2	H	H	OSO_3^-	+1	0.359
GTX3	H	OSO_3^-	H	+1	0.638
GTX4	OH	OSO_3^-	H	+1	0.726
R4=$CONHSO_3^-$ (n-sulfocarbamoyl (sulfamate) toxins)					
C1	H	H	OSO_3^-	0	0.006
C2	H	OSO_3^-	H	0	0.096

Figure 1 Structure and relative toxicity of the saxitoxin class of cyanotoxins

Materials and methods

For all experiments except chlorination, saxitoxin spiking material was purified from a toxic scum of *Anabaena circinalis* from Victoria, Australia. This material had a toxin profile characteristic of Australian strains of *A. circinalis* (Velzeboer *et al*., 2000), i.e. *C*1 and *C*2 toxins predominated with lesser quantities of GTX2, GTX3 and saxitoxin (STX). Details are given by Newcombe (2002).

Analysis of toxins

Saxitoxins were determined by HPLC with post-column derivatisation and fluorescence detection using a technique modified from Oshima (1995). Details are given by Rositano *et al*. (1998).

Chlorination

Chlorination of saxitoxins was carried out with semi-purified algal material collected from a bloom in Coolmunda Dam, Warwick, Queensland. This material also had a toxin profile characteristic of Australian strains of *A. circinalis* (Velzeboer *et al*., 2000), Reservoir water was dosed with the semi-purified material, and then with sufficient chlorine to produce a residual of 0.5 mg L^{-1} after 30 minutes. The pH of samples was adjusted before chlorine dosing and the pH was measured after 30 minutes contact time. Because of the relatively large chlorine doses required, there was substantial pH changes during the experiments. The pH at the end of the experiments was taken as the reaction pH. Initial toxin concentrations ranged from approximately 175 μg L^{-1} for *C*1 through to 10 μg L^{-1} for saxitoxin itself.

Ozonation

Ozone stock solution was added to 250 mL of test solution and allowed to react for 5 minutes. Residual ozone was purged using nitrogen. Full details are given by Rositano *et al*. (2001).

Powdered and granular activated carbon application

Experiments using powdered activated carbon were undertaken as described by Cook *et al*. (2001). Granular activated carbon laboratory studies are described by Newcombe (2002).

Results and discussion

Chlorine

Destruction of saxitoxins by chlorine was dependent both on pH and the particular toxin (Figure 2). The order of ease of removal of the saxitoxins was STX > GTX3 ~ C2 > C1 > GTX2. A high removal was possible at pH 9 provided a residual of 0.5 mg L^{-1} free chlorine was present after 30 minutes contact time. Removal as a function of pH was not linear with the degree of removal increasing significantly at around pH 7.5. The more effective removal at higher pH was unexpected as chlorine is known to be a weaker oxidant under these conditions. However the more effective removal may be due to the toxin molecule being present in an unprotonated form at higher pH and therefore more susceptible to oxidation. This is supported by the fact that in the detection of saxitoxins using post-column oxidation, sensitivity, which depends on the oxidation of these toxins to fluorescent derivatives, increases as the pH increases from 6.5 (Sullivan *et al*., 1985). Thus oxidation, at least to form fluorescent derivatives, is more efficient as the pH increases.

The feasibility of using chlorine to remove saxitoxins will depend on the pH of the water, the chlorine dose, initial concentrations of toxins and the degree of removal required. Removal may be improved by pH adjustment. Although saxitoxins are not detected by chemical analysis after chlorination under optimum conditions, there is no indication

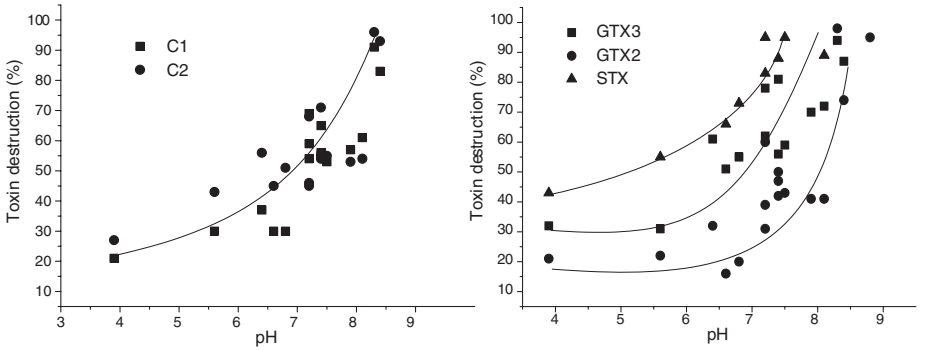


Figure 2 Percent saxitoxin destroyed by chlorine as a function of pH. Chlorine dose was sufficient to provide a 0.5 mgL^{-1} residual after 30 minutes

as to the nature of the oxidation products. However, mouse toxicity testing of chlorinated extracts showed that acute toxicity was also removed by chlorination which suggests that the products of this process are relatively benign.

Ozone

A mixture of saxitoxins was exposed to ozone by the addition of stock ozone solution into a batch reactor containing the water sample spiked with toxin. The solution was allowed to react for 5 minutes, after which time the ozone was removed by sparging with nitrogen. The experiment was carried out in three different treated waters. As seen in Figure 3, all of the toxins appear to be recalcitrant to oxidation by ozone, at ozone levels higher than those used in practice for disinfection.

Activated carbon adsorption

Both powdered and granular activated carbon (PAC and GAC) are effective in the removal of toxicity from a mixture of saxitoxins. Figure 4a shows the percent removal from a mixture of the toxins by five powdered activated carbons. As a general trend, the adsorption of the compounds decreases as STX > GTX > C. Although the charge of the compounds shows

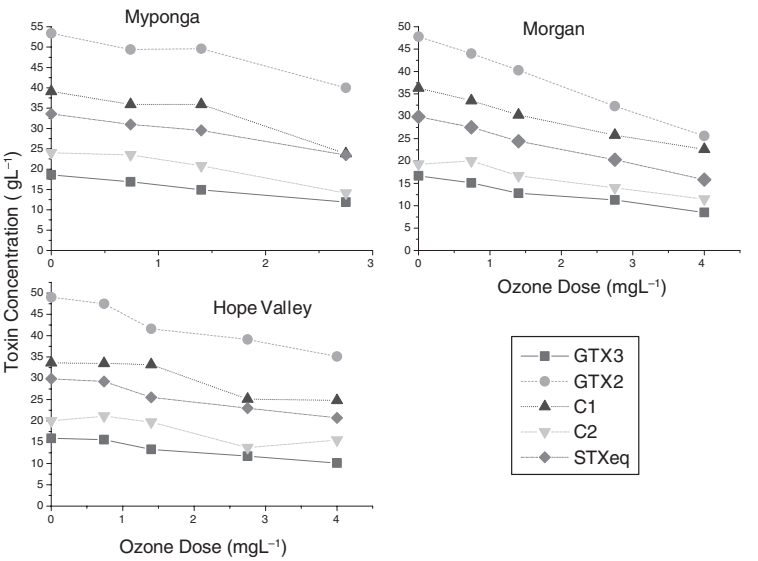


Figure 3 Saxitoxin concentration as a function of ozone dose

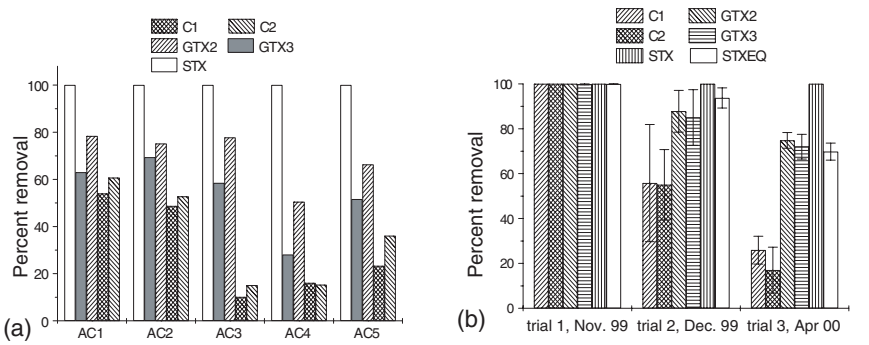


Figure 4 (a) Adsorption of saxitoxins onto 5 powdered activated carbons. (b) removal of saxitoxins by GAC during a 6 month investigation

a similar trend [STX(+2) > GTX(+1) > C(0)] this is unlikely to be the major effect as the carbon with the most positive surface charge (AC2) also displays the highest adsorption. The size of the compounds in solution follows the trend STX < GTX < C and it is likely that the relationship between the size of the compound and the pore volume distribution of the activated carbon plays the major role in adsorption. Figure 4b shows the percent removal of saxitoxins by the granular form of AC1 over a 6 month laboratory investigation. A mixture of saxitoxins was spiked into the influent water to the GAC column at the beginning of the 6 month investigation (trial 1), after 1 month (trial 2) and after 6 months (trial 3). The trends in removal are the same as illustrated in Figure 4a. After 6 months the removal of toxicity, measured in saxitoxin equivalents, was still satisfactory – approximately 70%.

Summary and conclusions

Chlorination is an effective process for the destruction of saxitoxins under conditions of high pH >8. Such pH adjustment may not be an attractive option in some water treatment plants.

Ozonation, up to ozone exposure values of 6.9 mg L^{-1} min, is not very effective for the destruction of the saxitoxins. Additional treatments are required.

In general it has been found that the more toxic saxitoxins are effectively adsorbed, therefore granular activated carbon, alone or in combination with ozone, and powdered activated carbon are viable treatment options. The studies indicate that GAC removes 70% of the toxicity of a saxitoxin solution after 6 months. It is difficult to extrapolate these results to longer GAC bed lives, or different mixtures of saxitoxins. However, all studies have shown that the most toxic, STX, is very readily removed by activated carbon.

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