

Removal of the cyanotoxin anatoxin-a by drinking water treatment processes: a review

Silvia Vlad, William B. Anderson, Sigrid Peldszus and Peter M. Huck

ABSTRACT

Anatoxin-a (ANTX-a) is a potent alkaloid neurotoxin, produced by several species of cyanobacteria and detected throughout the world. The presence of cyanotoxins, including ANTX-a, in drinking water sources is a potential risk to public health. This article presents a thorough examination of the cumulative body of research on the use of drinking water treatment technologies for extracellular ANTX-a removal, focusing on providing an analysis of the specific operating parameters required for effective treatment and on compiling a series of best-practice recommendations for owners and operators of systems impacted by this cyanotoxin. Of the oxidants used in drinking water treatment, chlorine-based processes (chlorine, chloramines and chlorine dioxide) have been shown to be ineffective for ANTX-a treatment, while ozone, advanced oxidation processes and permanganate can be successful. High-pressure membrane filtration (nanofiltration and reverse osmosis) is likely effective, while adsorption and biofiltration may be effective but further investigation into the implementation of these processes is necessary. Given the lack of full-scale verification, a multiple-barrier approach is recommended, employing a combination of chemical and non-chemical processes.

Key words | anatoxin-a, cyanobacteria, cyanotoxin, drinking water treatment, review

Silvia Vlad (corresponding author)
William B. Anderson
Sigrid Peldszus
Peter M. Huck
Department of Civil and Environmental
Engineering,
University of Waterloo,
200 University Ave. W.,
Waterloo, ON,
Canada,
N2L 3G1
E-mail: svlad@uwaterloo.ca

INTRODUCTION

Historically referred to as very fast death factor (Carmichael & Gorham 1978), the neurotoxic alkaloid anatoxin-a (ANTX-a) was first identified in the prairie region of Canada, and has subsequently been detected at low $\mu\text{g/L}$ concentrations in surface waters throughout North America, South America, Europe, Africa, Asia and New Zealand (Carmichael & Gorham 1978; Park *et al.* 1998; Ballot *et al.* 2003; Carrasco *et al.* 2007; Kotak & Zurawell 2007; Wood *et al.* 2007; Faassen *et al.* 2012; Ruiz *et al.* 2013). It can be produced by several genera of cyanobacteria, including *Anabaena*, *Oscillatoria*, *Cylindrospermum*, *Aphanizomenon*, and in some instances *Microcystis*, *Raphidiopsis*, *Arthrospira*, *Nostoc* and *Phormidium* (Osswald *et al.* 2007; Van Apeldoorn *et al.* 2007), and can co-occur with other cyanotoxins and/or taste and odour compounds produced by cyanobacteria (Ruiz *et al.* 2013). During the growth phase of cyanobacterial blooms cyanotoxins exist predominantly

intracellularly. However, weakening or rupture of the cell membrane due to bacterial ageing, physical stresses placed on the cells, or exposure to chemicals such as the oxidants used in drinking water treatment can all cause toxins to be released (Hart *et al.* 1998; WHO 1999; Ho *et al.* 2012).

The presence of cyanotoxins in drinking water sources has been a cause of concern as they have the potential to compromise public health, and many treatment processes have been investigated for their ability to remove the various classes of toxins which can be produced (Westrick *et al.* 2010; Merel *et al.* 2013b; Pantelić *et al.* 2013). The majority of studies on cyanotoxins and their fate during drinking water treatment have focused on microcystins and particularly the microcystin-LR variant (Merel *et al.* 2013b). The lack of information on treatment options for ANTX-a, and other cyanotoxins including cylindrospermopsin, nodularin and beta-methylamino-L-alanine, has been noted frequently

(WHO 1999; Hitzfeld *et al.* 2000; Westrick *et al.* 2010; Global Water Research Coalition 2012; Merel *et al.* 2013a). Of the studies undertaken into the treatment of ANTX-a, some have provided contradictory results and no comprehensive review has been presented to date to reconcile these results. All published studies have been at the bench- or pilot-scale, with no full-scale data available. This review aims to discern the necessary practices and operating parameters for optimal extracellular toxin removal, and highlights areas where further study is required to allow a treatment strategy to be implemented with confidence. Numerous studies have been published regarding the potential for cell lysis during water treatment, and operational considerations for removing intact cyanobacterial cells; however, removal of intracellular ANTX-a is not within the scope of this review.

ANALOGUES, CHEMICAL PROPERTIES AND STABILITY

ANTX-a is a relatively low molecular weight molecule (MW = 165) with a pK_a of 9.36; at pH levels relevant to drinking water (pH 6–9), it exists predominantly in the more stable protonated, cationic form, shown in Figure 1(a) (Van Apeldoorn *et al.* 2007). However, some portion of the toxin does exist in the neutral form (Figure 1(b)), as illustrated in Figure 2, which shows that at pH 6, ANTX-a is completely protonated (<1% deprotonated), while at pH 8.5, approximately 12% is deprotonated and at pH 9, 24% is deprotonated.

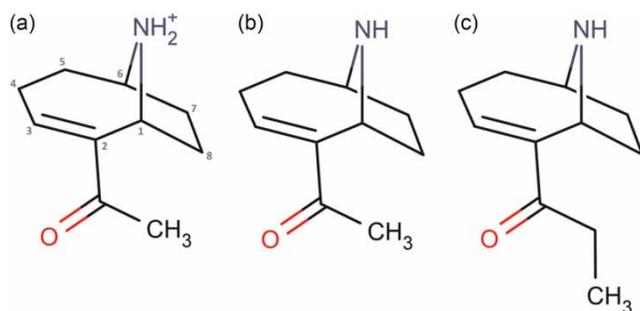


Figure 1 | Molecular structures: (a) protonated (+)-anatoxin-a stereoisomer (asymmetric centres at carbons 1 and 6, the secondary aminogroup for the (–)-anatoxin-a stereoisomer is located below the plane of the carbon ring); (b) deprotonated (+)-anatoxin-a; (c) homoanatoxin-a.

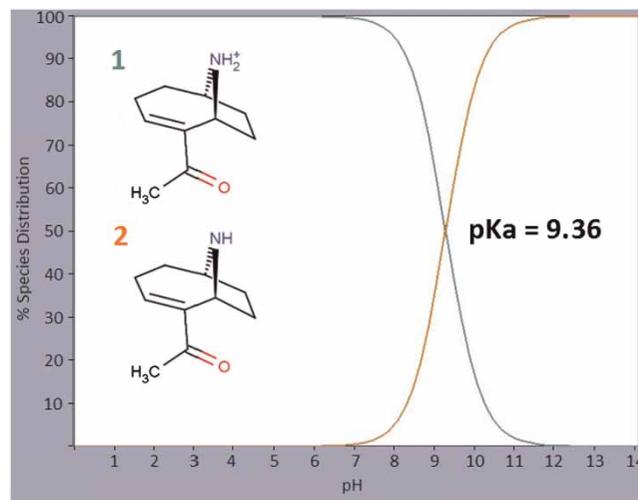


Figure 2 | Anatoxin-a species distribution based on pH. Figure produced with Marvin-Sketch software © 2013 by ChemAxon Ltd.

Kaminski *et al.* (2013) indicated that increasing the pH of a solution accelerated the decomposition of ANTX-a under a variety of conditions including increased temperature (100 °C) and ultraviolet (UV)-B exposure. They noted that at acidic pH (3.5), 1 hour exposure to 100 °C temperature or 36 $\mu\text{mol m}^{-2} \text{s}^{-1}$ UV-B radiation both caused minimal degradation of ANTX-a (<10%), while the same treatments at pH 9.5 resulted in a toxin reduction of nearly 80%. It should be noted, however, that the initial toxin concentration used in this study (25 mg/L) is several orders of magnitude greater than would typically be detected in natural waters, as will be discussed subsequently. These results imply that pH can be a critical factor in the treatment of ANTX-a, however, most studies do not differentiate between the treatment efficiency for the protonated and deprotonated forms of the toxin.

Conflicting results exist regarding the decomposition of ANTX-a in direct sunlight; Stevens & Krieger (1991) found that sunlight accelerates decay kinetics (1–2 hour half-lives observed), while Kaminski *et al.* (2013) observed only 3% toxin degradation following 5 hours of irradiation with photosynthetically active radiation (1,500 $\mu\text{mol m}^{-2} \text{s}^{-1}$). However, in dark conditions (as in most drinking water treatment plants and distribution systems) the toxin can persist for weeks or months (Stevens & Krieger 1991; Van Apeldoorn *et al.* 2007; Yang 2007).

It should be noted that of the two stereoisomers of the ANTX-a molecule, only (+)-ANTX-a is produced naturally

as a cyanobacterial metabolite (Valentine *et al.* 1991); this isomer is the more potent of the two forms of the toxin, with over ten times greater toxicity than (–)-ANTX-a (Adeyemo & Sirén 1992; Valentine *et al.* 1991). The (+)-ANTX-a stereoisomer is shown in Figure 1, with the asymmetric centres at carbons 1 and 6. In the (–)-ANTX-a stereoisomer, the secondary amino group is located beneath the plane of the carbon ring. It should be noted that most commercially available ANTX-a standards contain both stereoisomers in a racemic (50–50%) mixture.

One commonly identified analogue of ANTX-a is known as homoanatoxin (MW = 179), and differs from ANTX-a by one additional methyl unit on the side chain (Figure 1(c)). Although homoanatoxin is also highly neurotoxic (Wonnacott & Swanson 1992; Watanabe *et al.* 2003; Faassen *et al.* 2012), it is less frequently detected, and its treatment has not been investigated; this review therefore focuses on treatment of the more common analogue ANTX-a.

Anatoxin-a(s), while similar in name, is not structurally related to ANTX-a and homoanatoxin-a. This compound should not be confused with ANTX-a and its treatment is not considered in this review.

TOXICITY, OCCURRENCE AND REGULATIONS

ANTX-a is a nicotinic agonist whose toxicity has been well documented, resulting in a number of animal deaths globally (Edwards *et al.* 1992; Hitzfeld *et al.* 2000; Cadel-Six *et al.* 2007; Puschner *et al.* 2008; Environment Canada – Manitoba Water Stewardship 2011; Faassen *et al.* 2012). It is an acutely toxic compound with an LD₅₀ of 380 µg/kg (i.p. mouse) (Valentine *et al.* 1991), known to cause muscular paralysis and death due to respiratory arrest; however, the effects of chronic, low-level exposure are unknown, particularly with regard to human and animal reproduction (Osswald *et al.* 2007).

When detected, ANTX-a generally occurs at low concentrations environmentally (below 5 µg/L) (Robert *et al.* 2005; Fristachi *et al.* 2008); however, concentrations of up to 156 µg/L have been reported and in one case, a concentration of approximately 10 µg/L was detected in a post-treatment drinking water in Florida (Burns 2005). Based on current knowledge of the toxicity, potential health effects

and frequency of detection of ANTX-a, some regulatory bodies have set maximum concentrations for ANTX-a in drinking water (Table 1) and in recreational waters (Chorus 2012). Furthermore, a 1 µg/L drinking water guideline value has been recommended in the scientific literature, calculated to provide a safety margin of three orders of magnitude to protect against adverse effects of sub-lethal doses (Fawell *et al.* 1999). Although no regulations exist in the USA, ANTX-a is one of three cyanotoxins on the USEPA Candidate Contaminant List 3 (USEPA 2012), indicating a need for further study into its occurrence, effects and treatment, and the potential for forthcoming regulations.

WATER TREATMENT PROCESSES FOR EXTRACELLULAR ANATOXIN-A REMOVAL OR DEGRADATION

Oxidation

The ability to oxidize ANTX-a is highly dependent on the type and dose of oxidant used, the pH and background water characteristics, and the contact time provided for the reaction to occur. Rodríguez *et al.* (2007a) demonstrated that second-order rate constants can accurately represent the relative reactivity of various oxidants with ANTX-a, and these constants therefore serve as a general guideline to implementation requirements. A compendium of rate constants reported in the literature for ANTX-a oxidation in ultrapure water is presented in Table 2. The apparent reaction rates observed in natural water applications will

Table 1 | Regulations and guidelines for anatoxin-a concentration in drinking water

Location	Regulation/ Guideline	Status	Source
Quebec (Canada)	3.7 µg/L	Provisional	Institut national de santé publique du Québec (2005)
Oregon (USA)	3 µg/L	Adopted guideline	Oregon Health Authority (2013)
New Zealand	6 µg/L	Provisional	New Zealand Ministry of Health (2008)

Table 2 | Second-order rate constants reported for the oxidation of anatoxin-a in ultrapure water

Oxidant	Rate constant - second order ($M^{-1} s^{-1}$)	Conditions	Source
Cl ₂	0.71	pH 7	Rodríguez <i>et al.</i> (2007b)
NH ₂ Cl	<1	pH 8	Rodríguez <i>et al.</i> (2007a)
ClO ₂	Low	pH 8	Rodríguez <i>et al.</i> (2007a)
O ₃	5.6×10^4	pH 7	Bernazeau <i>et al.</i> (1995); Bruchet <i>et al.</i> (1998)
O ₃	6.4×10^4	pH 8	Onstad <i>et al.</i> (2007)
O ₃	9.7×10^5	pH 9	Onstad <i>et al.</i> (2007)
AOP - OH [•]	3.0×10^9	pH 7	Onstad <i>et al.</i> (2007)
AOP - OH [•]	5.2×10^9	pH 4.5–9.5	Afzal <i>et al.</i> (2010)
MnO ₄ ⁻	2.3×10^4	pH 8	Rodríguez <i>et al.</i> (2007a)

differ from the values given in Table 2 due to water quality effects. Rate constants collected in Table 2 have been given for the overall oxidation of ANTX-a at a stated pH.

Based on the data in Table 2, AOPs are the most effective oxidation process for ANTX-a degradation with a second-order rate constant four orders of magnitude greater than that reported for any other oxidant ($5.2 \times 10^9 M^{-1} s^{-1}$). Ozone has the second highest rate constant for ANTX-a oxidation, and appears particularly effective at higher pH, with the rate constant increasing by an order of magnitude between pH 8 and pH 9. Permanganate has a similar (but slightly lower) rate constant to ozone, while the chlorine-based oxidants (chlorine, chloramines and chlorine dioxide) all have very low rate constants (less than $1 M^{-1} s^{-1}$). These rate constants are consistent with redox potential of the various oxidative species considered, which is a fundamental indicator of the oxidizing power of each chemical, and ranks the oxidants as follows: OH[•] radical > ozone > permanganate > chlorine dioxide > chlorine (Zhou & Smith 2002). The relative reactivity with ANTX-a established by these constants is largely supported by the toxin degradation studies carried out to date.

Compared to other cyanotoxins, ANTX-a is relatively recalcitrant to oxidation. The second-order rate constants with ozone, OH[•] radicals and chlorine are all higher for both microcystin-LR and cylindrospermopsin than for ANTX-a (Acero *et al.* 2005; Onstad *et al.* 2007; Rodríguez *et al.* 2007a, 2007b), although the second-order rate constant for ANTX-a oxidation with permanganate ($2.3 \times 10^4 M^{-1} s^{-1}$) is several orders of magnitude higher than those for

microcystin-LR ($357 M^{-1} s^{-1}$) and cylindrospermopsin ($0.9 M^{-1} s^{-1}$) (Rodríguez *et al.* 2007a).

Numerous studies have reported some degree of ANTX-a oxidation, and their results, as well as the relevant factors listed above, are summarized in Table 3. However, little consideration has been given to the possible creation of toxic by-products from these oxidation reactions, and degradation of the parent toxin may not always be synonymous with reduction of overall toxicity. Complete mineralization of micropollutants is usually not achievable under conditions employed in water treatment practice, and therefore further investigation is required into the intermediate products of this toxin with the various oxidants. Monitoring of the toxicological endpoint in addition to the reduction of the parent compound may allow conclusions to be drawn regarding the toxicity reduction, without the need to examine individual intermediates. This approach has been employed for oxidative treatment of hormones using a screening assay to determine changes in oestrogenic activity (Huber *et al.* 2004), and may be appropriate for cyanobacterial toxicity studies. However, the possibility that the by-products of ANTX-a oxidation may have a different toxicological effect should also be considered.

Chlorine

It has been established repeatedly that chlorine is ineffective for the treatment of ANTX-a (Keijola *et al.* 1988; Hart *et al.* 1998; Hall *et al.* 2000; Newcombe & Nicholson 2004; Rodríguez *et al.* 2007a). A range of concentrations, contact times

Table 3 | Percent anatoxin-a degradation reported for various water treatment oxidants

Oxidant	% Toxin reduction	pH	Water matrix ^a	Oxidant dose (mg/L)	Contact time (min)	Initial anatoxin concentration (µg/L)	Detection limit (µg/L)	Study
Cl ₂	16	6–7	N	15 (4.5 residual)	30	20	5	Newcombe & Nicholson (2004)
Cl ₂	15	8	N (DOC 3.6 mg/L)	3	–	166	–	Rodríguez <i>et al.</i> (2007a)
Cl ₂	8	7	N (DOC 6.7 mg/L)	3	24 h	165	–	Rodríguez <i>et al.</i> (2007b)
Cl ₂	0	–	N	0.5	–	22 ^b	–	Keijola <i>et al.</i> (1988)
O ₃	46	7	U	1.8	3	1,000	0.2	Al Momani (2007)
	63	11	U	2.0	–	1,000	–	
O ₃	>90	–	T	2.0	–	2.4–4.3	–	Hall <i>et al.</i> (2000)
O ₃	92	7	T	0.11 residual (after 60 s)	–	24	–	Rositano <i>et al.</i> (1998)
O ₃	96	–	N	1	–	22 ^b	–	Keijola <i>et al.</i> (1988)
O ₃	>95	7.9	N (DOC 1.6 mg/L)	0.8	30	150	–	Onstad <i>et al.</i> (2007)
	95	7.2	N (DOC 13.1 mg/L)	>2.0	30	150	–	
O ₃	95	8	N (DOC 3.6 mg/L)	0.75	–	166	–	Rodríguez <i>et al.</i> (2007a)
O ₃	100	7.8	N (DOC 5.3 mg/L)	1.1	5	20	0.01	Rositano <i>et al.</i> (2001)
	100	7.5	N (DOC 4.6 mg/L)	1.7	5	20	–	
	100	7.8	N (DOC 5.7 mg/L)	1.5	5	20	–	
	100	7.1	N (DOC 15.5 mg/L)	>2.2	5	20	–	
AOP – H ₂ O ₂ /Fe(II)	100	7	U	0.1 Fe(II), 0.02 H ₂ O ₂	1.5	1,000	0.2	Al Momani (2007)
AOP – O ₃ /H ₂ O ₂	>98	7	U	0.001 H ₂ O ₂ , 2.0 O ₃	3	1,000	0.2	Al Momani (2007)
	100	7	U	0.01 H ₂ O ₂ , 1.0 O ₃	3	1,000	–	
AOP – O ₃ /Fe(II)	85	7	U	1.0 O ₃ , 0.5 Fe(II)	3	1,000	0.2	Al Momani (2007)
AOP – LP UV/ H ₂ O ₂	70	7	D	250 mJ/cm ² UV	–	600	33	Afzal <i>et al.</i> (2010)
				30 mg/L H ₂ O ₂				
AOP – VUV	>95	7	D	96 mJ/cm ²	–	600	33	Afzal <i>et al.</i> (2010)
	85	6.9	S (TOC 6.63 mg/L)	192 mJ/cm ²	–	600	–	
	70	7.3	N (TOC 3.94 mg/L)	192 mJ/cm ²	–	600	–	
AOP – TiO ₂ /UV	100	–	U	1% m/v slurry TiO ₂ ; (UV unspecified)	30	5,000	–	Robertson <i>et al.</i> (1999)
MnO ₄ [–]	>90	–	T	2.0	–	2.4–4.3	–	Hall <i>et al.</i> (2000)
MnO ₄ [–]	100	8	N (DOC 3.6 mg/L)	0.5	–	166	–	Rodríguez <i>et al.</i> (2007a)

^aWater matrix classification: U, ultrapure; T, treated drinking water; N, natural; D, deionized, S, synthetic/modelled.

^bInfluent concentration approximated based on cyanobacterial biomass.

– Indicates parameter not reported.

and pH levels relevant to drinking water treatment have been examined in various natural water sources (as detailed in Table 3) resulting in a consensus within the scientific literature that the oxidation of ANTX-a by chlorine is too slow a process to be considered an effective treatment barrier. This is a significant finding as most other cyanotoxins are well oxidized by this standard disinfectant.

Conventional treatment plants employing chlorine as primary disinfectant may therefore be facing a greater risk from ANTX-a. Although, in general, the water industry is moving away from using chlorine for this purpose, many jurisdictions in the world require the maintenance of a disinfectant residual (typically chlorine or chloramines) in the distribution system. While plants doing this could rely on this application of chlorine at the end of the treatment process to remove other cyanotoxins, this step would not contribute to the removal of ANTX-a.

Chloramines and chlorine dioxide

Similarly to chlorine, chloramines cannot effectively oxidize the ANTX-a molecule, and the reaction rate is prohibitively slow with an apparent second-order rate constant of less than $1 \text{ M}^{-1} \text{ s}^{-1}$ (Rodríguez *et al.* 2007a). Minimal oxidation of ANTX-a is observed in the presence of chlorine dioxide; indeed, the reactivity is so low that the second-order rate constant is not measurable (Rodríguez *et al.* 2007a). As a result of these findings, neither of these disinfectants is considered a feasible barrier to ANTX-a in drinking water treatment, nor to the other common microcystins and cylindrospermopsin (Merel *et al.* 2010; Westrick *et al.* 2010).

Ozone

Ozone is believed to selectively attack both the double bond and the deprotonated amine moiety of the ANTX-a molecule (Onstad *et al.* 2007) and it has been shown that the two species have different reaction rates with the deprotonated form reacting somewhat faster with molecular ozone than the protonated form (Onstad *et al.* 2007) (Figure 3). It can be challenging to distinguish changes in degradation efficiency due to differences in reaction rates between the species from the increased degradation of the deprotonated form at high pH values. In addition, as discussed below,

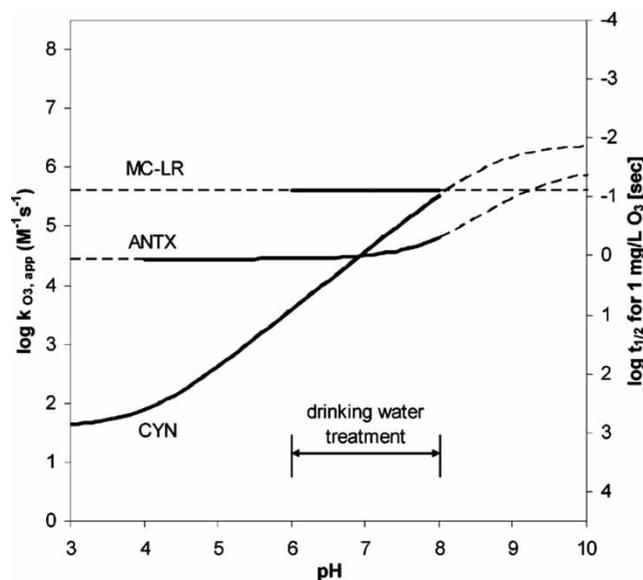


Figure 3 | pH dependence of the rate constants for the reaction of cyanotoxins with ozone. Half-life times are given for an ozone concentration of 1 mg/L. MC-LR, microcystin; CYN, cylindrospermopsin; ANTX, anatoxin-a. Reprinted with permission from Onstad *et al.* (2007) Copyright 2007 American Chemical Society.

ozone degradation produces OH radicals, which occurs more readily at higher pH values. This will also impact the observed reduction in ANTX-a concentrations during ozonation as it reacts more readily with OH radicals than molecular ozone.

Several studies have investigated the use of ozone for treatment of ANTX-a; while most have reported good to excellent toxin degradation, the degree of oxidation has been variable in some cases. These findings imply that optimal operating conditions have to be determined carefully in order to achieve a reliable reduction in ANTX-a toxicity utilizing ozone.

In ultrapure water, a 0.11 mg/L ozone residual after 60 seconds contact time has been shown to reduce ANTX-a concentrations of 24 µg/L by 92%, although the initial dose required to achieve that residual was not noted (Bernazeau *et al.* 1995; Rositano *et al.* 1998). In another study, 63% toxin reduction was achieved from a starting concentration of 1,000 µg/L ANTX-a, with ozone doses up to 2 mg/L, ozone residuals were not given, also in ultrapure water (Al Momani 2007). The extremely high initial ANTX-a concentration used in this study should be noted, as the lower percentage of ANTX-a degraded can be explained by examining the reactants on the basis of molar ratios. An

additional issue regarding this study is the lack of ozone residual data which is needed to estimate inactivations (e.g., CT calculations).

Further supporting the case for ozone use, high reductions have been attained in natural waters. Using 1 mg/L ozone, Keijola *et al.* (1988) achieved complete toxin elimination from an approximate starting concentration of 22 µg/L in natural water, although the water quality was undisclosed. In a lake water with 1.6 mg/L of dissolved organic carbon (DOC), an ozone dose of 0.8 mg/L was capable of oxidizing over 95% of ANTX-a (Onstad *et al.* 2007), and results reported by Hart *et al.* (1998) and Hall *et al.* (2000) showed greater than 90% toxin reduction with a 2 mg/L dose, from 2.4 to 4.3 µg/L initial toxin concentrations in treated natural water. Although the Hart *et al.* (1998) and Hall *et al.* (2000) results are very encouraging, the kinetic parameters such as the contact time were not reported, and there was no indication of what processes were used to prepare the 'treated' water matrix.

In more organic-rich raw waters, toxin degradation diminishes severely, as expected for ozone operation; at the same 2 mg/L O₃ dose approximately 50% toxin degradation was noted by Hall *et al.* (2000). Furthermore, Onstad *et al.* (2007) showed that in waters with high organic content (13.1 mg/L DOC), ozone doses above 2 mg/L were required to oxidize 95% of ANTX-a. These results indicate a scavenging effect from other natural water constituents and emphasize the importance of appropriate, source-specific pretreatment leading up to the oxidation process.

More conclusively, Rositano *et al.* (2001) endeavoured to determine the effects of different background water characteristics on ozone oxidation of ANTX-a by applying the oxidant to four toxin-spiked natural waters with varying properties, including DOC (4.6–15.5 mg/L), alkalinity (30–133 mg/L as CaCO₃), pH (7.1–7.8) and specific UV absorption (SUVA) (140–210 cm⁻¹ (mg/L)⁻¹ at 254 nm). They noted that complete elimination of the influent toxin (20 µg/L) was attainable, and relied on the maintenance of an ozone residual of approximately 0.05–0.06 mg/L after 5 minutes (CT = 0.25 mg min/L), which in three of the four waters, was attainable with ozone doses below 2 mg/L. Moreover, they found that in each of the waters examined, complete oxidation of ANTX-a required a higher ozone dose than was necessary for the

destruction of an equal influent concentration of microcystin-LR. The findings for ANTX-a degradation in natural water and the associated ozone dose requirements are consistent with the reported second-order rate constants for microcystin-LR, cylindrospermopsin and ANTX-a (Onstad *et al.* 2007).

The efficiency of the ozone–ANTX-a reaction has been shown to be pH dependent with better toxin degradation achieved at higher pH for the same dose (Al Momani 2007; Onstad *et al.* 2007). At pH 8, Onstad *et al.* (2007) reported an apparent second-order rate constant of $6.4 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ for the oxidation of ANTX-a (combined species) with ozone, which increased to $9.7 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ at pH 9 (Table 2). The observed pH dependence for ANTX-a degradation by ozonation is likely the result of three effects co-occurring: first, the degradation of molecular ozone increases at higher pH, thereby forming more hydroxyl radicals which are more reactive towards ANTX-a than molecular ozone as indicated by their second-order rate constants presented in Table 2; further, the deprotonated toxin is less stable and degradation of this species may be another removal pathway; finally, at high pH, the proportion of the deprotonated neutral ANTX-a species increases, which is more reactive than the protonated form with a reported second-order rate constant of $8.7 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ (Onstad *et al.* 2007). The last of these effects is well illustrated by Figure 3, reproduced from Onstad *et al.* (2007), which shows the rate constants for molecular ozone with three cyanotoxins, and their pH dependence. This study used a radical scavenger to suppress any reactions with OH radicals in order to measure the rate constants for molecular ozone alone which are illustrated in Figure 3. ANTX-a is evidently more reactive at higher pH values with this oxidant.

Disinfection by-product formation as a result of oxidant doses required for toxin degradation is another factor which needs to be considered when implementing ozonation. Rodríguez *et al.* (2007a) found only 1 µg/L bromate formation when ozone was applied to a natural water with 50 µg/L spiked bromide, for complete oxidation of 165 µg/L influent ANTX-a. However, the oxidation intermediates which may be produced from the ozone–anatoxin reaction sequence remain to be investigated, and the potential toxicity of those intermediate species is unknown.

Overall, most studies agree that ozone has the ability to oxidize ANTX-a effectively, provided oxidant scavenging by background natural organic matter (NOM) constituents, pH and temperature are duly accounted for. As with most ozone applications, the precise dose and contact time required to achieve the desired level of toxin degradation will depend upon local water quality and treatment conditions, but published material can serve as a guide for expected operation. The poorer performance (only 63% toxin degradation) observed by Al Momani (2007), was from a study which employed extremely high ANTX-a concentrations (several orders of magnitude above those normally observed in natural waters); studies which examined more environmentally relevant toxin concentrations all achieved greater than 90% oxidation. Ozone doses typically employed in drinking water disinfection (less than 2 mg/L) appear to be appropriate for this application, and greater than 99.9% (3-log) degradation of ANTX-a is predicted for systems meeting the ozone dose and contact time requirements for *Escherichia coli*, viruses, and *Giardia lamblia* disinfection (Onstad *et al.* 2007). However, care should be taken to validate attainment of the expected toxin degradation.

Advanced oxidation processes (AOPs)

AOPs are considered an effective treatment for many micro-pollutants and emerging contaminants, including cyanotoxins, due to the non-specificity of the oxidizing hydroxyl radical (Jin *et al.* 2012; Lee & von Gunten 2012). The second-order rate constants for the reaction of the hydroxyl radical with organic micropollutants are typically very fast, i.e., in the range of $10^9 \text{ M}^{-1} \text{ s}^{-1}$ and provided that hydroxyl radicals present in excess reaction rates are essentially diffusion controlled. However, when employing AOPs in natural water, hydroxyl radicals are not necessarily available in excess. Hydroxyl radical formation and concentrations are dependent on the operating parameters employed for a particular AOP and on the natural water matrix. Various AOPs have been studied for their ability to reduce ANTX-a concentrations, including $\text{O}_3/\text{H}_2\text{O}_2$, UV/ H_2O_2 , the Fenton process, $\text{O}_3/\text{Fe(II)}$ and titanium dioxide photocatalysis. Generally, $\text{O}_3/\text{H}_2\text{O}_2$ and UV/ H_2O_2 have been employed at full-scale for other contaminants in

drinking water treatment whereas the other processes have been applied either in groundwater remediation or at bench-scale.

ANTX-a degradation efficiency is increased by the addition of OH· radical-promoting chemicals to an ozone process, such as hydrogen peroxide (H_2O_2) or ferrous ions (Fe(II)). In ultrapure water hydrogen peroxide and ozone combined can achieve 100% elimination of ANTX-a in less than 180 seconds, with increased H_2O_2 doses (0.001–0.01 mg/L) increasing degradation efficacy (Al Momani 2007), although this H_2O_2 dose (0.1 mg/L with 1 mg/L ozone) was relatively low – an approximate stoichiometric ratio of 0.3 g H_2O_2 :1 g O_3 is more typical (Crittenden *et al.* 2012). The increased degradation observed was likely due to enhanced formation of hydroxyl radicals. In the $\text{O}_3/\text{Fe(II)}$ process, addition of Fe(II) in excess of 0.5 mg/L did not result in improved toxin degradation, although at 1 mg/L ozone and 0.5 mg/L Fe(II), 85% toxin degradation was achieved (Al Momani 2007).

In the same study, use of the Fenton process [a combination of H_2O_2 and Fe(II)] also yielded complete oxidation of ANTX-a, with the H_2O_2 concentration controlling the total degradation, likely enhancing hydroxyl radical production, and an inverse relationship between pH and toxin degradation. With the application of 0.005 mg/L H_2O_2 and 0.1 mg/L Fe(II), the toxin degradation increased from 60% at pH 7 to 73% at pH 3, at a contact time of 180 s. This pH relationship is the opposite of what was observed for ozone, and is likely attributable to the fact that the Fenton process is more effective in producing hydroxyl radicals at acidic pH values (Chang *et al.* 2008). Of the AOPs investigated by Al Momani (2007), the Fenton process had the highest apparent (first-order) rate constant for oxidation of ANTX-a, followed by $\text{O}_3/\text{H}_2\text{O}_2$ and $\text{O}_3/\text{Fe(II)}$, with all of the AOPs studied showing much higher apparent rate constants than ozone alone. This relative reactivity is consistent with the difference in magnitude between the second-order rate constants for reactions with OH· radicals and molecular ozone as shown in Table 2. Similarly, preliminary work conducted by Robertson *et al.* (1999) with titanium dioxide photocatalysis showed complete toxin decomposition within 30 minutes. This was validated by comparison with toxin destruction under control conditions – illumination without the catalyst

present – which showed <5% degradation. The limited toxin degradation observed in this control (UV irradiation) is in agreement with the results discussed in the section on UV treatment.

Although the removals observed by Robertson *et al.* (1999) and Al Momani (2007) in ultrapure water are promising, no investigation was made in either study into the effects of natural water conditions; scavenging of the oxidants by NOM and the impact of excess alkalinity need to be considered when applying AOPs at full scale as a barrier to cyanotoxins. Furthermore, the ANTX-a concentrations used in both studies (0.5–5 mg/L) are several orders of magnitude higher than those relevant for naturally occurring blooms and therefore the applicability of these results to typical source waters needs to be assessed under natural water conditions.

Afzal *et al.* (2010) examined two UV-based AOPs – vacuum UV photolysis (VUV) at 172 nm and low pressure (LP) UV/H₂O₂ – and found both to be effective in the oxidation of ANTX-a. VUV was capable of reducing a 600 µg/L ANTX-a concentration to below the 33 µg/L detection limit (>95% removal) in deionized water; however, as expected, investigations in model and natural waters showed a reduction in the process efficiency, with only 70–85% removals observed for double the UV dose (Afzal *et al.* 2010). A similar trend was observed for the LP UV/H₂O₂ process, with decreased removals in natural and synthetic water, although only 70% toxin reduction was reported in deionized water. A possible dose–response relationship was observed between H₂O₂ dose and ANTX-a removal with UV/H₂O₂, up to a 40 mg/L dose (Afzal *et al.* 2010). As H₂O₂ alone does not appear to effectively oxidize ANTX-a, with only 5% reduction in toxin concentration reported by Al Momani (2007), this relationship is attributed to increased production of the OH· radical.

Table 2 presents the two pseudo-second-order rate constants which have been reported for the reaction of ANTX-a with the OH· radical: $3.0 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ at pH 7 (Onstad *et al.* 2007), and $5.2 \pm 0.3 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$. The latter was reported to be independent of pH (4.5–9.5) and temperature (8–48 °C) (Afzal *et al.* 2010). There is good agreement between these rate constants given that they were established independently by different labs using different methodologies.

Collectively these results indicate that although AOPs are capable of achieving complete removal of ANTX-a, the operating conditions and selection of the OH· radical generation process play critical roles in determining the toxin degradation efficacy and efficiency. Furthermore, all the studies presented in the literature have used initial concentrations of ANTX-a higher than would be expected in environmental conditions. The effects of oxidant scavenging in natural waters would likely be more severe for lower influent toxin concentrations, and this issue should be addressed by future studies.

Permanganate

Potassium permanganate has been used as an effective oxidative barrier to numerous emerging contaminants, including cyanotoxins (Hart *et al.* 1998; Hall *et al.* 2000; Rodríguez *et al.* 2007a). A 2 mg/L dose may be sufficient to reduce a starting ANTX-a concentration of 2.4–4.3 µg/L by over 90% in treated water, although the necessary contact time and pretreatment employed have not been indicated (Hall *et al.* 2000). Between pH 6 and 8, the reaction appears constant, but reactivity increases markedly between pH 8 and 10 (Rodríguez *et al.* 2007a). The apparent second-order rate constant is provided in Table 2, and indicates that permanganate is slightly less reactive with ANTX-a than ozone, and much less reactive than the hydroxyl radical. In natural water with 3.6 mg/L DOC, 0.5 mg/L permanganate was able to completely eliminate 166 µg/L influent toxin, indicating this oxidant is very well suited to treatment of ANTX-a, even in the presence of background NOM (Rodríguez *et al.* 2007a). Other studies have shown better removal with permanganate than ozone, an unexpected result based on the second-order rate constants (Hart *et al.* 1998; Hall *et al.* 2000; Rodríguez *et al.* 2007a). In the case of Rodríguez *et al.* (2007a), the authors attributed the higher removals to the impacts of the natural water matrix, indicating that it increased the stability of permanganate resulting in increased oxidant exposure.

Membrane filtration

Due to the small molecular size of ANTX-a, LP membranes (microfiltration and ultrafiltration) are generally not

considered a barrier to the dissolved, extracellular toxin, although they can be used effectively to remove intact cyanobacterial cells under appropriate operating conditions (Global Water Research Coalition 2012). As a result, this review considers only high pressure membranes, namely nanofiltration and reverse osmosis.

Based on molecular weight cut-offs (150–700 Da), nanofiltration can be a promising candidate for ANTX-a removal. At low molecular weight cut-offs <165 Da (i.e., the molecular weight of ANTX-a) complete removal would be expected based on size exclusion. But even where the molecular weight cut-off of a membrane (Trisep TS80 4040) is slightly higher than the molecular weight of the target compound (200 Da vs. 165 Da), removal efficiency of greater than 96% can be achieved indicating that mechanisms other than size exclusion are important for removal (Gijsbertsen-Abrahamse *et al.* 2006). ANTX-a adsorption to nanofiltration membranes has been observed in multiple studies (Gijsbertsen-Abrahamse *et al.* 2006; Teixeira & Rosa 2011, 2012), and therefore accurate removal values can only be determined after an appropriate acclimation period of at least 48 h. Although these effects were considered in the Gijsbertsen-Abrahamse *et al.* (2006) study, some limitations still apply to their results: in this bench-scale investigation, recovery was only 10% of the feed water, which is significantly lower than the >75% recoveries often employed in full-scale operation where multiple stages are employed. The initial ANTX-a concentration (4.6–4.8 µg/L) was within a relevant range for impacted source waters; however, with increased recovery, the feed water toxin concentration will increase and lower removals may result.

Teixeira & Rosa (2006) demonstrated removals up to 96.6% from an electrolyte solution spiked with 10 µg/L ANTX-a using a negatively charged nanofiltration membrane (Alfa Laval NFT50) operating with 10 bar transmembrane pressure and 0.91 m/s crossflow. While the molecular weight cut-off of the NFT50 (150 Da) is lower than the molecular weight of ANTX-a (165 Da), the two values are quite close and size exclusion was likely not the only mechanism for removal as observed removals changed with changes in operating conditions. Teixeira & Rosa (2006) found that lower pH (pH 4–8) and the presence of CaCl₂ both enhanced the achievable toxin removals. At low pH, addition of 1 mM CaCl₂ resulted in an increase in

removal efficiency from 83.3% (pH 4.3) to 96.6% (pH 4.0) and at higher pH the removal increased from 67.9% (pH 8.2) to 88.7% (pH 7.7) with the addition of calcium. High rejection (95%) continued to be achievable even in clarified natural waters with NOM present. At recovery rates of up to 90%, indeed, the presence of background organics, including other cyanotoxins, removed the pH dependence observed in electrolyte solution, which was attributed to increased steric hindrances thereby increasing toxin rejection.

Nanofiltration membranes appear to be an effective treatment process for ANTX-a, although the scarcity of studies indicates a need for validation of these results, in particular for removal by nanofiltration membranes with higher molecular weight cut-offs. It should be noted that although high removals of ANTX-a have been observed, removal of microcystins was higher still, as expected given the molecular size differential. Removals for the tighter reverse osmosis membranes would be expected to be complete, given the ability of those membranes to reject even monovalent ions in desalination processes; however, this has not yet been demonstrated.

UV irradiation

Unlike UV irradiation employed for disinfection and AOPs using UV to generate hydroxyl radicals for contaminant degradation, direct photolysis degradation of ANTX-a using UV irradiation requires very high fluences (doses) (Hall *et al.* 2000; Afzal *et al.* 2010) although studies differ on how high. A study published by Hall *et al.* (2000) showed that doses in the range of 20,000 mJ/cm² were required to achieve toxin degradation, although toxin removal values were not given in this study, and the type of lamp used (medium vs. LP UV) was not noted. As the maximum absorbance for ANTX-a occurs at 227 nm, LP UV emitting only at 254 nm has been deemed ineffective (Afzal *et al.* 2010). Medium pressure (MP) lamps emit light over a wide wavelength spectrum and in their study using MP lamps, Afzal *et al.* (2010) achieved 88% and 50% toxin reductions from starting concentrations of 0.6 and 1.8 mg/L, respectively, using a UV fluence of 1,285 mJ/cm². Higher fluences will have to be employed to achieve removals greater than 90% which is well above the 20–100 mJ/cm² range typically employed for

disinfection in drinking water treatment (Crittenden *et al.* 2012).

As the Afzal *et al.* (2010) study was conducted in ultrapure water at bench-scale, and although fluence determinations take the character of the particular water characteristics into account, it is expected that the reported removals will be impacted by the presence of shielding and competing compounds in full-scale operations. Overall, this process is not an effective treatment barrier for ANTX-a as the high fluences required are economically unfeasible.

Adsorption

As was previously noted, ANTX-a exists both intra- and extracellularly, with the toxin predominantly found within intact cells during the bloom growth phase until cell lysis occurs as a result of ageing or other water treatment processes. While activated carbon may not be appropriate for the treatment of toxins bound within the intact cells, it may play a role in the removal of extracellular ANTX-a.

Activated carbon adsorption is considered a primary treatment barrier for many extracellular cyanotoxins (microcystin-LR,-RR,-YR, cylindrospermopsin, saxitoxin, etc.), although its effectiveness for removal of ANTX-a is less well understood (Cook & Newcombe 2002; Carrière *et al.* 2010). Few studies have investigated the use of powdered or granular activated carbon (PAC or GAC) for ANTX-a control and no adsorption isotherm parameters have been reported in the literature to date. The type of carbon used can strongly impact the removals obtained, as carbons produced from varying source materials (coconut, coal, wood, etc.) and activated via diverse techniques (steam activated, chemically activated, etc.) can exhibit different characteristics including surface charge and pore size distribution. Based on the properties of a target compound, a more microporous or more mesoporous carbon may be optimal for different microcontaminants, and based on background water characteristics, some carbons may perform better when the competitive and preloading effects of NOM are considered.

To manage the lack of available research results, some authors have suggested using microcystin adsorption as an indicator of potential ANTX-a removal (Carrière *et al.* 2010; Global Water Research Coalition 2012). However,

given the differences in chemical structures, charge, and molecular weight, microcystin may not be an appropriate surrogate for the adsorption behaviour of ANTX-a (Newcombe & Nicholson 2004), and impacts of NOM competition and carbon properties may be quite different for the two toxins. ANTX-a has a molecular weight of 165 Da, and is therefore more comparable in size to 2-methylisoborneol (MIB) (MW = 168 Da) and geosmin (MW = 182 Da) – two taste and odour compounds produced by cyanobacteria – than to microcystin (MW = 995 Da, microcystin-LR variant). Given the much greater availability of data regarding the removal of geosmin and MIB, these compounds may potentially serve as appropriate gauges of the feasibility of activated carbon processes for treatment of ANTX-a. Activated carbon is also seen as a primary barrier against taste and odour compounds, with high removals potentially achievable, and further study into the ANTX-a application is therefore warranted.

Granular activated carbon

Some preliminary work has been conducted using GAC, although the studies were limited in scope and results were somewhat contradictory. Hart *et al.* (1998) used a coal-based GAC rapid small scale column with an empty bed contact time of 6 minutes, and achieved greater than 90% initial removal but found 80% breakthrough of the 8.2 µg/L influent after 35,000 bed volumes (equivalent to approximately 18 weeks' operation). However, no information was given regarding the water matrix used, nor was any consideration given to competitive effects. Another study also noted ANTX-a removal using GAC at pilot scale, but was unable to distinguish between removals due to adsorption and the possibility of biological degradation within the active biofilm (UKWIR 1996).

Powdered activated carbon

Due to the seasonal and sporadic nature of blooms in many parts of the world, PAC is a more popular adsorptive treatment method for cyanobacterial metabolites (i.e., geosmin and MIB) as it allows operators greater flexibility and suffers less from the effects of pre-loading of carbon sites than does GAC. However, during the bloom growth phase much of the

toxin present may be contained intracellularly. The usefulness of PAC applied at the front end of the plant (as is typical) would therefore be limited, as only the extracellular toxin fraction at the plant intake would be accessible. Intracellular ANTX-a would be unaffected by the PAC, but could be released as the result of cell lysis in a later stage of the treatment train.

PAC removal studies for ANTX-a have also been limited: two studies reported 50–60% removal of ANTX-a using a 5 mg/L PAC dose, one from an influent with approximately 22 µg/L anatoxin (Keijola *et al.* 1988), and the other from an unspecified influent concentration (Bruchet *et al.* 1998). In both studies, contextual information was lacking: no information was given on the type of PAC, contact time or water matrix investigated.

A more promising result showed 90% toxin removal from 10 µg/L ANTX-a influent, but required 11 mg/L PAC, and 60 mg/L was required to remove 98% of ANTX-a from a 50 µg/L influent, although the contact time allotted was again unspecified (Mouchet & Bonnelye 1998). In contrast, typical PAC doses employed in drinking water treatment would be in the 5–25 mg/L range (Crittenden *et al.* 2012). The total organic carbon (TOC) of the water matrix studied varied between 4 and 6 mg/L, and therefore the adsorption process included competition from background organics, but only one carbon type (F400 coal based, Calgon Carbon) was considered, and as discussed above, carbon selection is a critical consideration for effective adsorption of micropollutants.

A systematic optimization of this treatment process could yield favourable results, but further information is required to be able to accurately and confidently determine PAC doses and contact times, based on the background water NOM concentration and composition, temperature, carbon type and influent toxin concentration.

BIOFILTRATION/SLOW SAND FILTRATION

Similarly to adsorption, despite being considered one of the more effective treatment processes for removal of cyanotoxins from drinking water, there is limited information available on the use of biofiltration or slow sand filtration as a barrier to dissolved ANTX-a (Ho *et al.* 2012). ANTX-a

degrading microorganisms seemingly occur naturally in various lake environments (Rapala *et al.* 1994), although only one such organism – a *Pseudomonas* sp. Gram-negative bacteria – has been identified (Kiviranta *et al.* 1991). The ANTX-a degradation rate in natural waters via this bacterium was determined to be 6–30 µg/mL over a 3 day period.

Rapala *et al.* (1994) noted biodegradation of ANTX-a in a batch sediment experiment, but only following a 4 day lag-phase; studies examining removal of other cyanotoxins – primarily microcystin-LR – using biofiltration have shown that an extended lag phase (up to 16 days) can be required prior to establishment of degrading microorganisms within the biofilm (Cousins *et al.* 1996; Ho *et al.* 2012), and based on the above findings, similar concerns may be anticipated for ANTX-a. As such, biodegradation may be limited to application in warmer climates where cyanobacterial blooms are a continual issue, rather than a seasonal and episodic concern as in more temperate regions.

There is a lack of information on studies dealing directly with degradation of the toxin within a biofilm, and no bench-, pilot-, or full-scale biofiltration studies are reported in the peer-reviewed literature. In the only study directly dealing with drinking water treatment processes, Keijola *et al.* (1988) reported 68–74% reduction of the neurotoxin from *Anabaena flos-aquae* culture (presumed to be ANTX-a), using pilot-scale slow sand filters with an approximate empty bed contact time of 100 minutes, and implicated biological activity by microscopic verification of the presence of biofilm.

There is evidence for the potential for biodegradation-based processes to contribute to a multiple-barrier treatment approach for ANTX-a, albeit the effects of temperature, empty bed contact time and background water quality remain unknown. Further validation of achievable removals within different operating conditions is required to confidently rely on biofiltration or slow sand filtration processes.

OTHER TREATMENT OPTIONS

Bank filtration intakes may provide ancillary benefits for cyanotoxin removal; Klitzke *et al.* (2011) noted that ANTX-a sorption to sediments was higher for clay- and organic-rich sediments than to sandy sediments, and reported various

Langmuir sorption model parameters based on 10 different sediment types. This study may serve as a guide for the type of ANTX-a removals which could be achieved in a bank-filtration scenario, considering local soil and sediment compositions.

The majority of treatment options studied and presented in this review are intended to represent municipal-scale operations; as such, a knowledge gap exists for technologies implemented at smaller scale. While high-pressure membrane filtration and oxidation processes can be expected to perform similarly to those operations detailed above, other technologies such as home filtration devices – including both ion exchange and activated carbon filtration – are not well characterized, and their performance with respect to ANTX-a removal is therefore unknown. No studies have examined home carbon filtration devices for ANTX-a control and further investigation of the prospects of these

systems may be of value to owners of small-community or point-of-entry/point-of-use systems.

To the authors' knowledge, no studies have been published examining the use of ion exchange for removal of ANTX-a. *Monosov et al. (2012)* found that microcystin-LR was well removed but suffered from competitive effects of background NOM concentrations, and similar concerns can be expected for ANTX-a. Although due to the molecular differences between the two toxins, as discussed above, it would be inapt to extrapolate specific operating parameters such as resin types.

BEST PRACTICES

Physical removal via high-pressure membrane filtration and chemical elimination via oxidants such as ozone, AOPs and

Table 4 | Summary of treatment process efficacy for anatoxin-a removal

Process	Treatment barrier efficacy	Operational considerations/Comments
Membranes – NF/RO	Likely effective	Adsorption of toxin on membrane surface occurs, rejection efficiency governed by electrostatic interactions and steric hindrance, 90% recovery achievable However, no RO data to support theoretical rejection and only some NF membranes have been investigated. NF membranes with higher molecular weight cut-offs may not be as effective and need to be investigated
Oxidation – Ozone	Effective	1–2 mg/L doses resulted in >90% degradation in six studies, dependent on water quality
Oxidation – Permanganate	Effective	Efficiency increases above pH 8, 0.5–2 mg/L doses resulted in >90% degradation in two studies
Oxidation – AOPs	Effective	OH [•] generation process impacts efficiency, efficacy needs to be quantified for lower toxin concentrations and in natural water
Adsorption – PAC/GAC	May be effective	Not frequently investigated, effects of carbon type unknown, competition from background NOM may reduce efficacy (high PAC doses and short GAC run times may be required)
Biofiltration	May be effective	Not frequently investigated, temperature, pH, water quality effects unknown, lag phase may occur prior to establishment of anatoxin-degrading species (may not be suitable for seasonal cyanobacterial outbreaks)
Oxidation – Chlorine	Ineffective	Reaction is prohibitively slow ($k_{app} < 1 \text{ M}^{-1} \text{ s}^{-1}$ at pH 8)
Oxidation – Chloramine	Ineffective	Reaction is prohibitively slow ($k_{app} < 1 \text{ M}^{-1} \text{ s}^{-1}$ at pH 8)
Oxidation – Chlorine dioxide	Ineffective	Reaction is prohibitively slow
UV	Ineffective at disinfection doses	Very high fluences required (1,285–20,000 mJ/cm ²)
Home filtration (POU) devices (Carbon)	Unknown	Further research needed
Ion exchange	Unknown	Further research needed

permanganate are the only processes which can presently be considered effective for the treatment of extracellular ANTX-a from drinking water, as summarized in Table 4. Various studies have validated the ability of each of the above treatments to remove or degrade over 90% of the toxin, and the impacts of diverse treatment conditions including different background water quality parameters such as TOC and pH have been reported and are considered manageable. While treatment with membranes, ozone and permanganate has been investigated at environmentally relevant toxin concentrations, the studies examining the various AOPs have dealt with high initial ANTX-a levels. Furthermore, often only one or two studies have been published on the different hydroxyl radical-producing processes, as Table 3 demonstrates; therefore, while AOPs are considered effective, further validation is recommended.

Conventional plants which employ none of these processes may be more vulnerable to this toxin than other cyanobacterial toxins including the more prevalent microcystins. In a survey of treatment plants in the Canadian province of Quebec, it was projected that under a climate change scenario (15 µg/L influent ANTX-a concentrations) no plants using chlorine as the exclusive treatment barrier would be able to conform to the 3.7 µg/L provisional provincial guideline (Carrière *et al.* 2010).

Among the physical and chemical processes discussed, some challenges remain to be considered, including the potential for toxic by-product formation in oxidative treatment and the management of residuals produced in high-pressure membrane treatment. Due to the uncertainty associated even with those treatment processes considered effective for ANTX-a removal, and the lack of full-scale data, a multiple treatment barrier approach is recommended to ensure that public health is safeguarded and that potential future regulations and guidelines can be met. A combination of oxidation and physical or sometimes biological removal has been employed for other cyanotoxins; however, in the absence of applicable information for activated carbon and biofiltration, non-chemical processes for ANTX-a are limited to high-pressure membrane filtration.

It was noted in several instances that important information was missing to assist with the interpretation of some studies. Table 5 presents suggestions for parameters which should be monitored and included in published

Table 5 | Recommended anatoxin-a study parameters

Study type	Parameters
Oxidation – Cl ₂ , O ₃ , AOPs, Permanganate	CT (time of exposure, oxidant residual at time of anatoxin-a sample collection and at T_0 – if applicable), pH, temperature
UV-based (including AOPs)	UV-fluence/dose, type of lamp (LP/MP), reactor configuration, if MP wavelengths are blocked below 200 nm, water flow rate, pH, temperature
PAC	Carbon type (porosity/pore size distribution, source material, particle size, surface charge), contact time, type and dose of coagulant used (if any), pH, temperature
GAC	Hydraulic loading, empty bed contact time, media/carbon type (as above), effective size, uniformity coefficient, media depth, pH, temperature
Biofiltration	Hydraulic loading, empty bed contact time, media/carbon type (as above), effective size, uniformity coefficient, media depth, pH, temperature, biomass parameters e.g., ATP
Studies considering natural waters	Alkalinity, pH, temperature, TOC, DOC, UV ₂₅₄ , SUVA, turbidity, NOM

materials, to allow drinking water practitioners to gauge the re-applicability of study results to similar conditions.

CONCLUSIONS

Further work is needed to ascertain the conditions under which the treatment processes discussed herein can be applied successfully at full-scale for the removal of ANTX-a. Oxidation via permanganate or ozone, and AOPs have all been repeatedly identified as processes effective for ANTX-a treatment, but especially for AOPs, studies in natural water and at environmentally relevant ANTX-a concentrations are still required. There is a consensus that UV, chlorine, chloramine and chlorine dioxide have been acknowledged as ineffective under drinking water treatment

conditions. High pressure membrane filtration is likely effective, with excellent results for some nanofiltration membranes, although removals for NF membranes with larger molecular weight cut-offs need to be validated; furthermore, the assumption that reverse osmosis membranes will be capable of achieving high rejection of ANTX-a has not yet been substantiated. Preliminary results for activated carbon adsorption (particularly PAC) and biofiltration show good potential, but indicate a need for further investigation, while the potential of other processes or process combinations, including ion-exchange and home treatment units, remains unknown. A multiple-barrier approach is suggested for utilities impacted by this toxin, to mitigate the uncertainties related to process optimization.

ACKNOWLEDGEMENTS

Funding for this project was provided by the Natural Sciences and Engineering Research Council of Canada (NSERC) in the form of an Industrial Research Chair in Water Treatment at the University of Waterloo. The current Chair partners include: the Region of Waterloo, Walkerton Clean Water Centre, City of Brantford, City of Hamilton, Lake Huron and Elgin Area Water Supply Systems, Region of Halton, City of Ottawa, City of Guelph, Region of Niagara, Region of Durham, City of Barrie, City of Toronto, Ontario Clean Water Agency (OCWA), Conestoga-Rovers & Associates Ltd, Associated Engineering Group Ltd, GE Water & Process Technologies Canada, MTE Consultants Inc., EPCOR Water Services, and RAL Engineering Ltd. This work was produced as part of a project conducted in partnership with the Walkerton Clean Water Centre.

REFERENCES

- Acero, J. L., Rodriguez, E. & Meriluoto, J. 2005 Kinetics of reactions between chlorine and the cyanobacterial toxins microcystins. *Water Res.* **39** (8), 1628–1638.
- Adeyemo, O. M. & Sirén, A. L. 1992 Cardio-respiratory changes and mortality in the conscious rat induced by (+)- and (+/-)-anatoxin-a. *Toxicon* **30** (8), 899–905.
- Afzal, A., Oppenländer, T., Bolton, J. R. & El-Din, M. G. 2010 Anatoxin-a degradation by advanced oxidation processes: vacuum-UV at 172 nm, photolysis using medium pressure UV and UV/H₂O₂. *Water Res.* **44** (1), 278–286.
- Al Momani, F. 2007 Degradation of cyanobacteria anatoxin-a by advanced oxidation processes. *Sep. Purif. Technol.* **57** (1), 85–93.
- Astrachan, N., Archer, B. & Hilbelink, D. 1980 Evaluation of the subacute toxicity and teratogenicity of anatoxin-a. *Toxicon* **18**, 684–688.
- Ballot, A., Pflugmacher, S., Wiegand, C., Kotut, K. & Krienitz, L. 2003 Cyanobacterial toxins in Lake Baringo, Kenya. *Limnologica* **33** (1), 2–9.
- Bernazeau, F., Baudin, I., Pieronne, P., Bruchet, A. & Anselme, C. 1995 Traitement des problèmes des toxines générées par les algues. TSM. *Techniques Sciences Méthodes, Génie Urbain Génie Rural* **10**, 747–748.
- Bruchet, A., Bernazeau, F., Baudin, I. & Pieronne, P. 1998 Algal toxins in surface waters: analysis and treatment. *Water Supply* **16** (1/2), 611–623.
- Burns, J. 2005 CyanoHABs – the Florida experience. In International Symposium on Cyanobacterial Harmful Algal Blooms, 6–10 September, Durham, NC, USA.
- Cadel-Six, S., Peyraud-Thomas, C., Brient, L., de Marsac, N. T., Rippka, R. & Méjean, A. 2007 Different genotypes of anatoxin-producing cyanobacteria coexist in the Tarn River, France. *Appl. Environ. Microbiol.* **73** (23), 7605–7614.
- Carmichael, W. & Gorham, P. 1978 Anatoxins from clones of *Anabaena flos-aquae* isolated from lakes of western Canada. *Mitteilungen Internationale Vereinigung für Theoretische und Angewandte Limnologie* **21**, 285–295.
- Carrasco, D., Moreno, E., Paniagua, T., de Hoyos, C., Wormer, L., Sanchis, D., Cires, S., Martin-del-Pozo, D., Codd, G. A. & Quesada, A. 2007 Anatoxin-a occurrence and potential cyanobacterial anatoxin-a producers in Spanish reservoirs. *J. Phycol.* **43** (6), 1120–1125.
- Carrière, A., Prévost, M., Zamyadi, A., Chevalier, P. & Barbeau, B. 2010 Vulnerability of Quebec drinking-water treatment plants to cyanotoxins in a climate change context. *J. Water Health* **8** (3), 455–465.
- Chang, C.-Y., Hsieh, Y.-H., Cheng, K.-Y., Hsieh, L.-L., Cheng, T.-C. & Yao, K.-S. 2008 Effect of pH on Fenton process using estimation of hydroxyl radical with salicylic acid as trapping reagent. *Water Sci. Technol.* **58** (4), 873–879.
- Chorus, I. 2012 *Current Approaches to Cyanotoxin Risk Assessment Risk Management and Regulations in Different Countries*. Federal Environment Agency, Dessau-Roßlau, Germany. Available at: <http://www.uba.de/uba-info-medien-e/4390.html> (accessed February 25 2014).
- Cook, D. & Newcombe, G. 2002 Removal of microcystin variants with powdered activated carbon. *Water Sci. Technol.* **2** (5–6), 201–207.
- Cousins, I., Bealing, D., James, H. & Sutton, A. 1996 Biodegradation of microcystin-LR by indigenous mixed bacterial populations. *Water Res.* **30** (2), 481–485.
- Crittenden, J., Trussell, R. R., Hand, D. W., Howe, K. J. & Tchobanoglous, G. 2012 *MWH Water Treatment Principles and Design*, 3rd edn. John Wiley & Sons, Hoboken, NJ, USA.

- Edwards, C., Beattie, K. A., Scrimgeour, C. M. & Codd, G. A. 1992 Identification of anatoxin-A in benthic cyanobacteria (blue-green algae) and in associated dog poisonings at Loch Insh, Scotland. *Toxicon* **30** (10), 1165–1175.
- Environment Canada – Manitoba Water Stewardship 2011 State of Lake Winnipeg: 1999 to 2007. Report of Environment Canada. Ottawa, ON, Canada, pp. 1–209.
- Faassen, E. J., Harkema, L., Begeman, L. & Lurling, M. 2012 First report of (homo)anatoxin-a and dog neurotoxicosis after ingestion of benthic cyanobacteria in The Netherlands. *Toxicon* **60** (3), 378–384.
- Fawell, J. K., Mitchell, R. E., Hill, R. E. & Everett, D. J. 1999 The toxicity of cyanobacterial toxins in the mouse: II anatoxin-a. *Hum. Exp. Toxicol.* **18** (3), 168–173.
- Fristachi, A., Sinclair, J. L., Hall, S., Berkman, J. A. H., Boyer, G., Burkholder, J., Carmichael, W., DuFour, A., Frazier, W., Morton, S. L., Brien, E. O. & Walker, S. 2008 Occurrence of Cyanobacterial Harmful Algal Blooms Workgroup Report. In: *Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs* (K. H. Hudnell, ed.). Springer Press, Triangle Park, NC, USA, pp. 45–103.
- Gijbsbertsen-Abrahamse, A., Schmidt, W., Chorus, I. & Heijman, S. 2006 Removal of cyanotoxins by ultrafiltration and nanofiltration. *J. Membr. Sci.* **276** (1–2), 252–259.
- Global Water Research Coalition 2012 *International Guidance Manual for the Management of Toxic Cyanobacteria* (G. Newcombe, ed.). GWRC Report Series, IWA Publishing, London, UK.
- Hall, T., Hart, J., Croll, B. & Gregory, R. 2000 Laboratory-scale investigations of algal toxin removal by water treatment. *Water Environ.* **14** (2), 143–149.
- Hart, J., Fawell, J., Croll, B. & Marsalek, B. 1998 Algal toxins in surface waters: origins and removal during drinking water treatment processes. *Water Supply* **16** (1/2), 611–623.
- Hitzfeld, B. C., Höger, S. J. & Dietrich, D. R. 2000 Cyanobacterial toxins: removal during drinking water treatment, and human risk assessment. *Environ. Health Persp.* **108** (Supplement 1), 113–122.
- Ho, L., Sawade, E. & Newcombe, G. 2012 Biological treatment options for cyanobacteria metabolite removal – a review. *Water Res.* **46** (5), 1536–1548.
- Huber, M. M., Ternes, T. A. & von Gunten, U. 2004 Removal of estrogenic activity and formation of oxidation products during ozonation of 17alpha-ethinylestradiol. *Environ. Sci. Technol.* **38** (19), 5177–5186.
- Institut national de santé publique du Québec 2005 Propositions de critères d'intervention et de seuils d'alerte pour les cyanobactéries. Available at: <http://www.inspq.qc.ca/pdf/publications/348-CriteresInterventionCyanobacteries.pdf> (accessed December 23 2013).
- Jin, X., Peldszus, S. & Huck, P. M. 2012 Reaction kinetics of selected micropollutants in ozonation and advanced oxidation processes. *Water Res.* **46** (19), 6519–6530.
- Kaminski, A., Bober, B., Lechowski, Z. & Bialczyk, J. 2013 Determination of anatoxin-a stability under certain abiotic factors. *Harmful Algae* **28**, 85–87.
- Keijola, A. M., Himberg, K., Esala, A. L., Sivonen, K. & Hiisvirta, L. 1988 Removal of cyanobacterial toxins in water treatment processes: laboratory and pilot scale experiments. *Toxicity Assess.* **3**, 643–656.
- Kiviranta, J., Sivonen, K. & Lahti, K. 1991 Production and biodegradation of cyanobacterial toxins-a laboratory study. *Arch. Hydrobiol.* **121** (3), 281–294.
- Klitzke, S., Beusch, C. & Fastner, J. 2011 Sorption of the cyanobacterial toxins cylindrospermopsin and anatoxin-a to sediments. *Water Res.* **45** (3), 1338–1346.
- Kotak, B. G. & Zurawell, R. W. 2007 Cyanobacterial toxins in Canadian freshwaters: a review. *Lake Reserv. Manage.* **23** (2), 109–122.
- Lee, Y. & von Gunten, U. 2012 Quantitative structure-activity relationships (QSARs) for the transformation of organic micropollutants during oxidative water treatment. *Water Res.* **46** (19), 6177–6195.
- Merel, S., Clement, M. & Thomas, O. 2010 State of the art on cyanotoxins in water and their behaviour towards chlorine. *Toxicon* **55**, 677–691.
- Merel, S., Villarín, M. C., Chung, K. & Snyder, S. 2013a Spatial and thematic distribution of research on cyanotoxins. *Toxicon* **76**, 118–131.
- Merel, S., Walker, D., Chicana, R., Snyder, S., Baurès, E. & Thomas, O. 2013b State of knowledge and concerns on cyanobacterial blooms and cyanotoxins. *Environ. Int.* **59**, 303–327.
- Monosov, I., Bazri, M., Imoberdorf, G., Vazquez-Rodriguez, G. A., Barbeau, B. & Mohseni, M. 2012 Removal of cyanobacterial toxins and NOM using ion exchange resins. In *Proceedings, AWWA – Water Quality and Technology Conference*, November 2012, Toronto, Canada.
- Mouchet, P. & Bonnelye, V. 1998 Solving algae problems: French expertise and world-wide applications. *J. Water Supply Res. Technol.-AQUA* **47** (3), 125–141.
- Newcombe, G. & Nicholson, B. 2004 Water treatment options for dissolved cyanotoxins. *J. Water Supply Res. Technol.-AQUA* **53** (4), 227–239.
- New Zealand Ministry of Health 2008 Drinking-water Standards for New Zealand 2005 (Revised 2008) 1-163. Available at: <http://www.health.govt.nz/publication/drinking-water-standards-new-zealand-2005-revised-2008-0> (accessed December 23 2013).
- Onstad, G. D., Strauch, S., Meriluoto, J., Codd, G. A. & Von Gunten, U. 2007 Selective oxidation of key functional groups in cyanotoxins during drinking water ozonation. *Environ. Sci. Technol.* **41** (12), 4397–4404.
- Oregon Health Authority 2013 Oregon Drinking Water Services Best Management Practices for Harmful Algae Blooms (HABs) for Drinking Water Providers 1-3. Available at: <http://public.health.oregon.gov/HealthyEnvironments/DrinkingWater/Operations/Treatment/Documents/algae/BMP-HABs.pdf> (accessed February 25 2014).
- Osswald, J., Rellán, S., Gago, A. & Vasconcelos, V. 2007 Toxicology and detection methods of the alkaloid neurotoxin

- produced by cyanobacteria, anatoxin-a. *Environ. Int.* **33** (8), 1070–1089.
- Pantelić, D., Svirčev, Z., Simeunović, J., Vidović, M. & Trajković, I. 2013 Cyanotoxins: Characteristics, production and degradation routes in drinking water treatment with reference to the situation in Serbia. *Chemosphere* **91**, 421–441.
- Park, H., Kim, B., Kim, E. & Okino, T. 1998 Hepatotoxic microcystins and neurotoxic anatoxin-a in cyanobacterial blooms from Korean lakes. *Environ. Toxicol. Water Qual.* **13** (3), 225–234.
- Puschner, B., Hoff, B. & Tor, E. R. 2008 Diagnosis of anatoxin-a poisoning in dogs from North America. *J. Vet. Diagn. Invest.* **20** (1), 89–92.
- Rapala, J., Lahti, K., Sivonen, K. & Niemela, S. I. 1994 Biodegradability and adsorption on lake sediments of cyanobacterial hepatotoxins and anatoxin-a. *Lett. Appl. Microbiol.* **19**, 423–428.
- Robert, C., Tremblay, H. & Deblois, C. 2005 Cyanobactéries et cyanotoxines au Québec: suivi à six stations de production d'eau potable (2001–2003). Report of the Québec Ministère du Développement durable, de l'Environnement et des Parcs, pp. 1–65.
- Robertson, P. K. J., Lawton, L. A., Munch, B. & Cornish, B. 1999 The destruction of cyanobacterial toxins by titanium dioxide. *J. Adv. Oxid. Technol.* **4** (1), 20–26.
- Rodríguez, E., Onstad, G. D., Kull, T. P. J., Metcalf, J. S., Acero, J. L. & von Gunten, U. 2007a Oxidative elimination of cyanotoxins: comparison of ozone, chlorine, chlorine dioxide and permanganate. *Water Res.* **41** (15), 3381–3393.
- Rodríguez, E., Sordo, A., Metcalf, J. S. & Acero, J. L. 2007b Kinetics of the oxidation of cylindrospermopsin and anatoxin-a with chlorine, monochloramine and permanganate. *Water Res.* **41** (9), 2048–2056.
- Rositano, J., Nicholson, B. C. & Pieronne, P. 1998 Destruction of cyanobacterial toxins by ozone. *Ozone-Sci. Eng.* **20** (3), 223–238.
- Rositano, J., Newcombe, G., Nicholson, B. & Sztajn bok, P. 2001 Ozonation of NOM and algal toxins in four treated waters. *Water Res.* **35** (1), 23–32.
- Ruiz, M., Galanti, L., Ruibal, A. L., Rodriguez, M. I., Wunderlin, D. A. & Amé, M. V. 2013 First report of microcystins and anatoxin-a co-occurrence in San Roque Reservoir (Córdoba, Argentina). *Water Air Soil Pollut.* **224** (6), 1593.
- Stevens, D. & Krieger, R. 1991 Stability studies on the cyanobacterial nicotinic alkaloid anatoxin-a. *Toxicon* **29** (2), 167–179.
- Teixeira, M. R. & Rosa, M. J. 2006 Neurotoxic and hepatotoxic cyanotoxins removal by nanofiltration. *Water Res.* **40** (15), 2837–2846.
- Teixeira, M. R. & Rosa, M. J. 2011 Adsorption of microcystins and anatoxin-a on nanofiltration membranes. In *Proceedings, 6th IWA Specialist Conference on Membrane Technology for Water & Wastewater Treatment*. 4–7 October, International Water Association, Aachen, Germany.
- Teixeira, M. R. & Rosa, M. J. 2012 How does the adsorption of microcystins and anatoxin-a on nanofiltration membranes depend on their co-existence and on the water background matrix. *Water Sci. Technol.* **66** (5), 976–982.
- UKWIR 1996 Pilot Scale GAC Tests to Evaluate Toxin Removal. Report of the UK Water Industry Research.
- USEPA 2001 Creating a Cyanotoxin Target List for the Unregulated Contaminant Monitoring Rule. Report of the USEPA, Cincinnati, OH, USA.
- USEPA 2012 Contaminant Candidate List 3 – CCL. Water: Contaminant Candidate List. Available at: water.epa.gov/scitech/drinkingwater/dws/ccl/ccl3.cfm#ccl3 (accessed November 10 2013).
- Valentine, W. M., Schaeffer, D. J. & Beasley, V. R. 1991 Electromyographic assessment of the neuromuscular blockade produced in vivo by anatoxin-a in the rat. *Toxicon* **29** (3), 347–357.
- Van Apeldoorn, M. E., van Egmond, H. P., Speijers, G. J. & Bakker, G. J. I. 2007 Toxins of cyanobacteria. *Mol. Nutr. Food Res.* **51** (1), 7–60.
- Watanabe, M., Tsujimura, S. & Oishi, S. 2003 Isolation and identification of homoanatoxin-a from a toxic strain of the cyanobacterium *Raphidiopsis mediterranea* Skuja isolated from Lake Biwa, Japan. *Phycologia* **42** (4), 364–369.
- Westrick, J., Szlag, D. C., Southwell, B. J. & Sinclair, J. 2010 A review of cyanobacteria and cyanotoxins removal/inactivation in drinking water treatment. *Anal. Bioanal. Chem.* **397** (5), 1705–1714.
- WHO 1999 *Toxic Cyanobacteria in Water: A Guide to Their Public Health Consequences, Monitoring and Management*. World Health Organization, Geneva, Switzerland. Available at: http://www.who.int/water_sanitation_health/resourcesquality/toxicyanbact/en/ (accessed December 23 2013).
- Wonnacott, S. & Swanson, K. 1992 Homoanatoxin: a potent analogue of anatoxin-a. *Biochem. Pharmacol.* **43** (3), 419–423.
- Wood, S. A., Rasmussen, J. P., Holland, P. T., Campbell, R. & Crowe, A. L. M. 2007 First report of the cyanotoxin anatoxin-a from *Aphanizomenon flos-aquae* (cyanobacteria). *J. Phycol.* **43** (2), 356–365.
- Yang, X. 2007 Occurrence of the Cyanobacterial Neurotoxin, Anatoxin-a, in New York State Waters. PhD dissertation, State University of New York College of Environmental Science and Forestry, New York, USA.
- Zhou, H. & Smith, D. 2002 Advanced technologies in water and wastewater treatment. *J. Environ. Eng. Sci.* **1** (4), 247–264.

First received 16 January 2014; accepted in revised form 7 March 2014. Available online 31 March 2014