Removal of microcystin variants with powdered activated carbon

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Abstract Hepatotoxic microcystins resulting from cyanobacteria blooms occur in drinking water sources worldwide. Their removal in the water treatment process is essential if safe drinking water is to be produced. The presence of microcystins in raw water is problematic to the water treatment plant operator, as they are not removed by conventional water treatment practices (coagulation, flocculation, sedimentation and filtration). Powdered activated carbon (PAC) has been shown to have the potential to remove microcystin-LR; however, its success is dependent on the application of a sufficient dose for the production of water that is safe to consume. Currently there is no method available for determining PAC doses for the removal of microcystin-LR and little information exists on the removal of other microcystin analogues. In previous work the Homogeneous Surface Diffusion Model (HSDM) was successfully used to predict the adsorption of earthy–musty tastes and odours onto PAC. In this study, adsorption experiments were performed to determine whether the HSDM could be applied to two microcystin analogues: microcystin-LR (mLR) and microcystin-LA (mLA). The HSDM was used to predict the kinetics of mLR and mLA adsorption onto a wood-based PAC from a spiked sample of reservoir water. From this information PAC doses required to remove the toxic compounds were predicted for a range of conditions. These two variants were more difficult to remove than microcystin-RR (mRR) and microcystin-YR (mYR). The ease of removal was found to be in the following order: mRR > mYR > mLR > mLA.

Keywords Activated carbon; microcystins; water treatment

Introduction

Microcystins resulting from cyanobacteria blooms occur in drinking water sources worldwide. In Australia, the main source of microcystins is from the cyanobacterium *Microcystis aeruginosa*. Microcystins consist of seven amino acids, with the terminal amino acids joined to form a cyclic compound. They have a general structure of:

cyclo-(D-alanine – *X* – D-MeAsp – *Z* – Adda – D-glutamamate – Mdha)

X and *Z* are variable L amino acids, D-MeAsp is D-erythro-β-methylaspartic acid, Mdha is N-methyldehydroalanine and Adda is (2S,3S,8S,9S)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid. Structural variations have been reported in all seven amino acids, but most frequently with the substitution of L-amino acids at positions 2 and 4, demethylation of amino acids at positions 3 and/or 7 (Sivonen and Jones, 1999). More than 60 different hepatotoxic microcystins have been characterised (Sivonen and Jones, 1999). Clinical signs of hepatotoxicosis have been observed in field poisonings involving cattle, sheep, horses, pigs, ducks and other wild and domestic animals (Carmichael, 1992), and have been implicated as the major cause of death of dialysis patients in a clinic in Caruaru, Brazil in 1996 (Pouria *et al*., 1998). Microcystin removal in the water treatment process is essential if safe drinking water is to be produced.

Coagulation and filtration can remove microcystin that is bound within the algal cell (Chow *et al*., 1999; Drikas *et al*., 2001) but is ineffective against dissolved or extracellular toxin (Hoffman, 1976; Falconer *et al*., 1983; Keijola *et al*., 1988; Drikas *et al*., 2001). Early

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studies prior to the identification of the structure of microcystin analogues showed that at the laboratory and pilot plant scale addition of PAC reduced the toxicity (Hoffman, 1976; Falconer *et al*., 1983). Subsequent research has focused on the most common and toxic microcystin analogue, mLR.

Studies examining the removal of mLR have found PAC can readily adsorb this compound but its effectiveness depends on the PAC contact time, initial mLR concentration and type of PAC used. Wood-based PACs have been found to be the most effective PACs for mLR adsorption. PAC adsorption studies of mLR by Donati *et al*. (1994) and Schumann *et al*. (1997) showed that the amount of mLR adsorbed at equilibrium onto PAC directly related to the volume of mesopores (1 nm < width > 50 nm). Donati *et al*. (1993) found that a mesoporous wood-based PAC displayed superior mLR adsorption efficiency at equilibrium and at short contact times expected in a water treatment plant than less mesoporous coal- and coconut-based PACs. If the occurrence of microcystins in source water is sporadic, PAC can be a cost-effective option as it is applied only when required. However, its success is dependent on the application of a sufficient dose for the production of water that is safe to consume. Currently there is no method available for determining PAC doses for the removal of mLR and little information exists on the removal of other microcystin analogues.

The HSDM has been successfully applied to predict the adsorption of earthy–musty tastes and odours onto PAC (Huang *et al*., 1996; Gillogly *et al*., 1998; Cook *et al*., 1999). The aim of this research was to assess whether the HSDM could be applied to two microcystin analogues (mLR and mLA) and to compare their removal with two other common microcystin analogues. This information can be used to predict the PAC dose required to remove these toxins for a given water quality. This information will improve the ability of water treatment plants to successfully produce water that is safe to drink when microcystin-producing cyanobacterial blooms are present in the source water.

Materials and methods

Water samples

Water samples were filtered through a 0.2 μm cartridge filter and stored at 4°C until used. The water used in this study was taken from Hope Valley reservoir in Adelaide. The dissolved organic carbon content and UV absorbance at 254 nm were 9.9 mg/L and 0.339 cm^{-1} respectively.

Powdered activated carbons

PACs were supplied from PICA. PAC A is a wood-based chemically activated carbon while PAC B is a steam activated coal-based activated carbon. The carbons were dried at 105°C for 24 hours, cooled, and then stored in a desiccator.

Microcystins

Microcystin LA and LR were isolated from a natural bloom of *Microcystis aeruginosa* that occurred in South Australia over the summer of 1998–99. The bloom produced an approximately 50/50 mix of mLR/mLA with some other minor unidentified analogues also present. This mixture was used in the PAC adsorption experiments. The presence of mLR and mLA was confirmed by liquid chromatography mass spectrometry. Microcystin YR was supplied by Calbiochem, catalogue number 475819, and Microcystin RR was supplied by Alexis, catalogue number 350-043-C100.

Experimental procedures

Equilibrium isotherms

Raw water spiked with microcystin was added to clean dry Pyrex® bottles to a level allowing minimum headspace. PAC was added and the bottle sealed and then agitated for 3 days. A bottle with no PAC served as a control from which the initial toxin concentration was determined. Prior to analysis, samples were filtered through 0.45 µm membrane filters to remove the PAC.

Kinetics

Raw water was spiked with microcystin and 700 mL portions were added to 2 litre acrylic jars with each portion representing a different contact time with PAC. PAC (pre-wetted overnight) was added to samples mixed at 100 rpm on a six-paddle gang stirrer. PAC contact time was between 10–60 minutes. Prior to analysis, samples were filtered through 0.45 µm membrane filters to remove the PAC.

Predicting mLR and mLA removal using the HSDM

The HSDM was used to predict the kinetics of mLR and mLA adsorption. This was performed according to the method described by Cook *et al*. (1999). A detailed explanation of the HSDM is given elsewhere (Najm *et al*., 1991; Traegner and Suidan, 1989).

Analytical methods

Microcystin analysis

Microcystin analysis was carried out as outlined by Rositano (1996).

Results and discussion

Predicting mLR and mLA adsorption

The HSDM was used to predict the kinetics of mLR and mLA adsorption. This model has successfully predicted the kinetics of MIB and geosmin removal with PAC (Huang *et al*., 1996; Gillogly *et al*., 1998; Cook *et al*., 1999). The model requires experimental parameters obtained from both equilibrium and kinetic tests. Equilibrium and batch kinetic tests (Figures 1 and 2) show that mLR was more readily adsorbed than mLA. The percentage removal of mLR and mLA was found to be independent of the initial concentration of each compound. Several researchers have found this trend for earthy–musty taste and odours

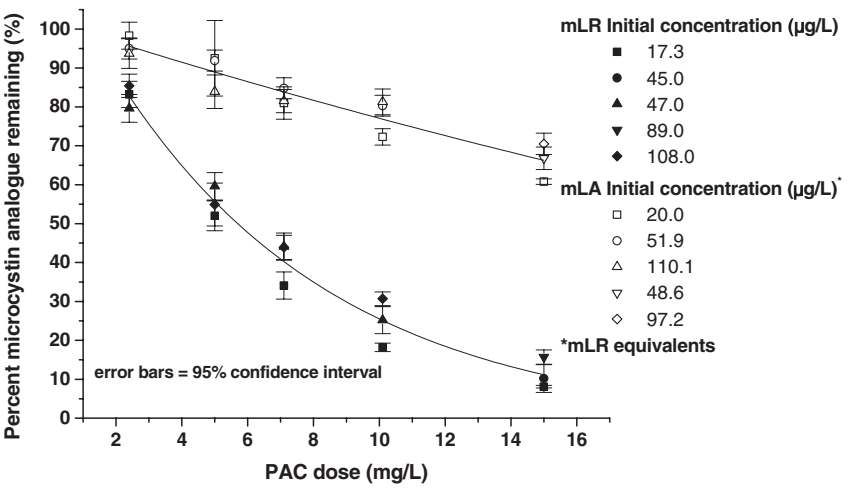

Figure 1 Equilibrium adsorption of mLR and mLA with PAC A

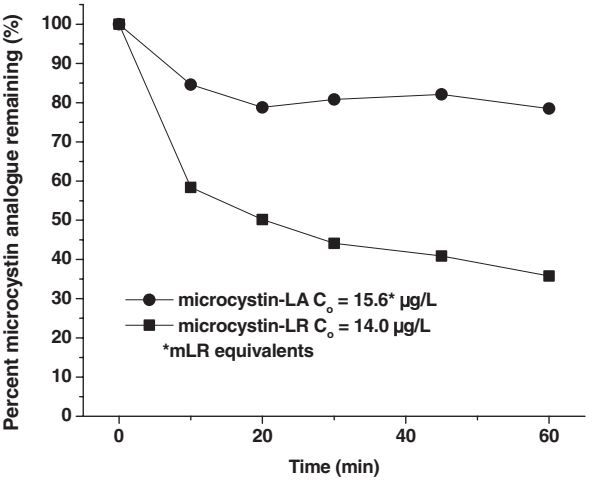

Figure 2 Percent removal of mLR and mLA with 15 mg/L of PAC A

and atrazine at concentrations applicable to water treatment (Gillogly *et al*., 1998; Knappe *et al*., 1998; Cook *et al*., 1999; Graham *et al*., 2000).

Figure 3 shows the HSDM fit (solid line) and HSDM prediction (broken line) for mLR and mLA. The difference between the mLR and mLA predicted removal and the experimental results was less than 10%, with most predictions within 1.5 µg/L of the experimental results. Using the kinetic and equilibrium parameters, the HSDM could be used to predict the PAC doses required to remove the toxins for a range of conditions (Table 1). The target concentration for each compound was 1 µg/L; the Australian Drinking Water Guideline value is 1.3 µg/L as mLR toxicity equivalents (NHMRC/ARMCANZ, 2001). For the reaction conditions used, PAC A would not be considered an option for mLA removal due to the large doses required.

Comparison of the adsorption of four microcystin analogues

The adsorption of other microcystin analogues was also investigated. It is important to investigate the adsorption of analogues other than mLR as it is seldom the only variant

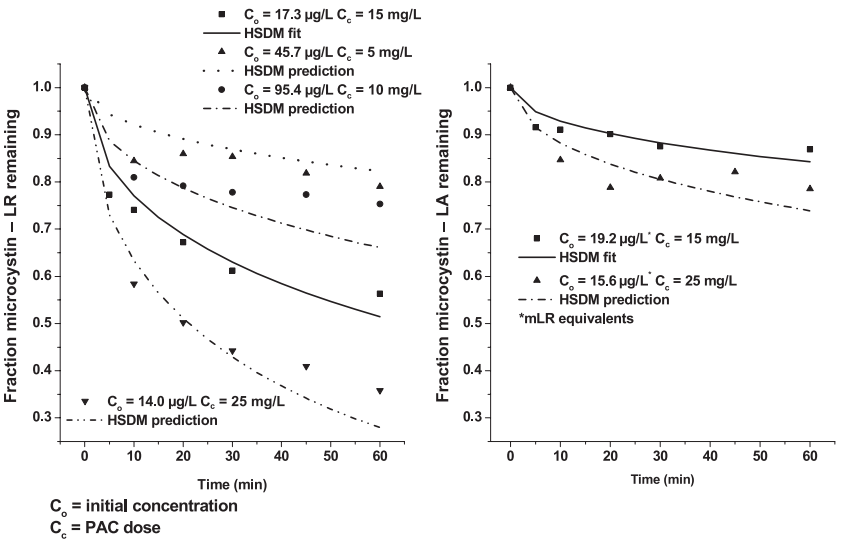

Figure 3 HSDM fit and prediction of mLR (left) and mLA (right) with PAC A

Table 1 Predicted PAC doses for the removal of mLR and mLA to 1 (g/L

Initial toxin concentration (μg/L)	MLR		mLA	
	Contact time (min)			
	30	60	30	60
	Predicted PAC dose (mg/L)			
2	23	18	>50	49
5	40	29	>50	>50
10	50	38	>50	>50

present, and is often not the most abundant. Table 2 gives the LD_{50} [i.p. mouse (μg/kg)] (Sivonen and Jones, 1999), molecular weight and number of charged groups for the four analogues. For the pH range (≈6–8.5) expected in water treatment the negative charged groups result from the dissociated carboxyl groups (COO^-) on D-glutamamate and D-erythro-β-methylaspartic acid. The positive charge is due to the variable amino acid, arginine that has a basic amino group (NH^+). These differences may result in different adsorption characteristics for each analogue. The only study to compare the adsorption of different microcystin analogues onto activated carbon was by Lawton *et al*. (1998). It was found that from a mixture of four microcystin analogues the percentage removal of mLR was approximately 0–10% lower than the more hydrophobic microcystins (mLY, mLW, and mLF) using domestic jug filters that incorporated a mixture of granular activated carbon and ion-exchange resin. Based on chemical structure, UKWIR (1997) estimated that the adsorption of other microcystin analogues by activated carbon to be similar or better than mLR. These estimates were based on computer model estimates of the octanol-water partition coefficient (K_{ow}) of each analogue.

Figure 4 shows that from a mixture of four microcystin analogues consisting of mRR, mYR, mLR and mLA, the ease of removal was mRR > mYR > mLR > mLA for both PACs tested. For equivalent concentrations toxin removal varied significantly with the variant. For example, 15 mg/L of PAC A after 30 minutes removed 90% of mRR but only 5% of mLA. The same trend was also evident at equilibrium (contact time = 3 days). The adsorption behaviour shown here does not correlate with estimates of log K_{ow} reported in the literature. Based on log K_{ow} reported by UKWIR (1997), mLA would be adsorbed to a greater extent than the other three analogues since it had the highest log K_{ow}, while the K_{ow} values for mRR and mYR were lower than mLR. This fits into the adsorption behaviour found here, but differs from that reported by Rivasseau *et al*. (1998). It was found that the retention factor (k_w) for mRR, mYR and mLR (pH7) on octadecysilica with water as the eluent, an indirect measurement of K_{ow}, to be very similar. These results show that the adsorption behaviour of microcystin analogues can not be predicted based on mLR adsorption data or the K_{ow} of each analogue.

The difference in adsorbability of the variants is currently under investigation. Differences in adsorption are likely to be due to a number of factors, such as the conformation or size of the microcystin molecule in solution and interaction between the toxin

Table 2 Properties of microcystins studied

Analogue	Variable amino acids		LD_{50}	Molecular weight	Charge at pH 6–8.5
	X	Z			
mRR	arginine	arginine	600	1,037	0 (– – & + +)
mYR	tyrosine	arginine	70	1,044	–1 (– – & +)
mLR	leucine	arginine	50	994	–1 (– – & +)
mLA	leucine	alanine	50	909	–2 (– –)

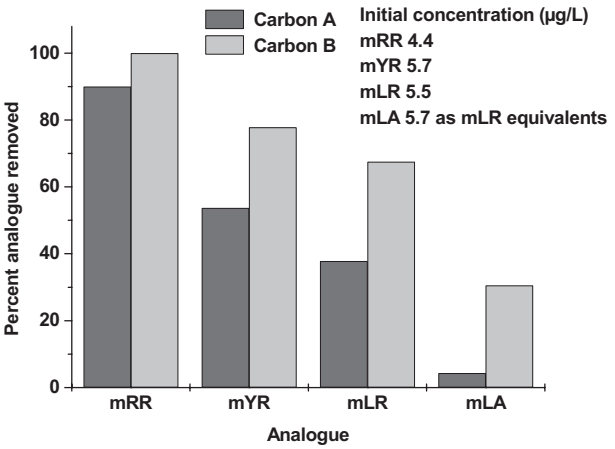

Figure 4 Percent removal of the four analogues with each PAC

molecule and the activated carbon surface. This may be with the bare activated carbon surface and/or with adsorbed toxin and natural organic matter. The degree to which these factors affect adsorption would be related to variable amino acid groups present on each analogue. A smaller conformation would favour adsorption, as there would be access to more adsorption sites on the activated carbon. Attraction or repulsion forces between the toxin molecule and the activated carbon surface could enhance or hinder adsorption.

Conclusion

The adsorption of mLR and mLA onto PAC was successfully predicted using the HSDM. This allowed prediction of the required PAC doses for removal of mLR and mLA in the water treatment process. This information will improve the ability of a water treatment plant to successfully produce water that is safe to drink when microcystin-producing cyanobacterial blooms are in the water supply. Successful application of the HSDM to mLR and mLA means that it should be applicable to other microcystin analogues.

The adsorption efficiencies onto PAC of four microcystin analogues were quite different with the ease of removal being of the order: mRR > mYR > mLR > mLA. This indicates that knowledge of the analogues present is necessary to determine the appropriate PAC dose to successfully treat the source water.

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