Human exposure to cyanotoxins: Exploring *in vitro* detoxification using atmospheric cold plasma treatment to protect human health

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Introduction

Recently, there have been increases in freshwater Harmful Algal Blooms (HABs) globally. HABs are known to contaminate some of the Great Lakes such as Lake Erie, which supplies over 80% of surface water in North America. HABs can produce cyanotoxins, many of which are hepatotoxic such as the microcystins (MCs) and Nodularin (NOD). The health effects of these include; promotion of various cancers, neurotoxicity, genotoxicity and potential carcinogenicity. Human exposure occurs through numerous pathways, with the major route being through ingestion of contaminated water and recreational use of water bodies. Current methods utilised by water treatment facilities to remove cyanobacteria and cyanotoxins from drinking water can be successful if tailored to individual toxins. However, with certain cyanobacterial species capable of producing more than one class of cyanotoxin, as well as possibly producing numerous congeners, their removal could become more problematic, posing a risk to consumers. Therefore, there is a need to try and effectively detoxify drinking water contaminated with cyanotoxins to safeguard human health by use of new and novel techniques, such as atmospheric cold plasma treatment (ACPT). To investigate this, six MCs, NOD, cylindrospermopsin (CYN), anatoxin-A (ATX-A) and the marine toxin domoic acid (DA) were subjected to ACPT, using helium gas and a helium molecular oxygen gas admixture.

Sample Analysis: UPLC-MS/MS

Analysis was carried out on an ACQUITY UPLC i-Class system coupled to a Xevo TQ-MS Mass Spectrometer (Waters, Manchester, UK), operated in ESI^+ mode. Detection and quantification was carried out using Multiple Reaction Monitoring (MRM). Separation of the MCs and NOD was achieved using an ACQUITY UPLC BEH C18 column, with CYN, DA and ATX-A separated using an ACQUITY UPLC HSS T3 column, both with column dimensions of 100 mm x 2.1 mm i.d., 1.8 µm particle size (Waters, UK) and maintained at 45°C. The mobile phases comprised of water and acetonitrile (ACN), both containing 0.1% formic *v/v*.

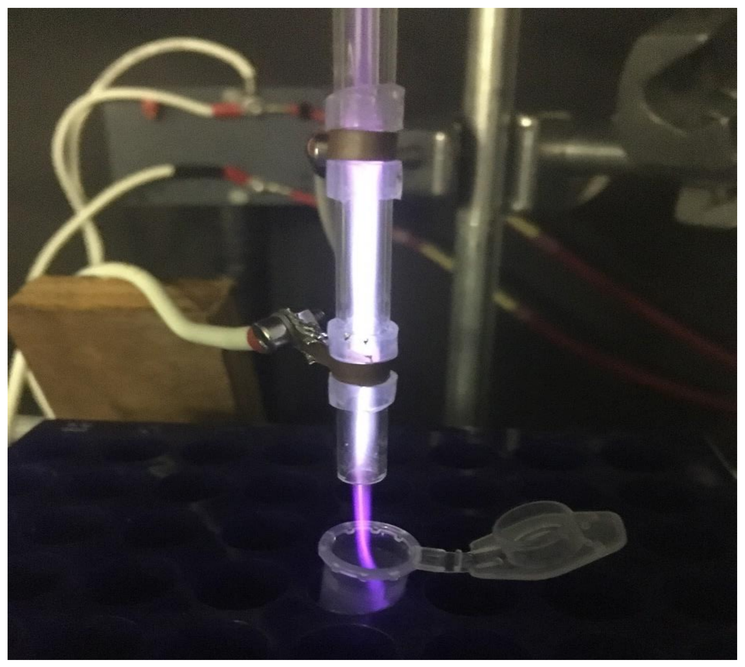

Figure 1. Image of the cold plasma treatment set-up using helium gas, with the plasma jet at a distance of 17 mm from the eppendorf tube.

Atmospheric Cold Plasma treatment (ACPT)

Eppendorfs containing a 1 mL solution of a multi-toxin standard were prepared in triplicate at a concentration of 0.25 µg/mL. The operating conditions consisted of a 5 kV source operated at 20 kHz and a gas flow rate of 2 standard litre per min (SLM) using helium (He) and an Helium/Oxygen admixture. Samples were subject to the plasma jet at a distance of 17 mm from the end of the quartz tube to the eppendorf, illustrated in Figure 1. Solutions were subject to zero (control), 5, 10, 20, 40 and 60 min of treatment, after which, solutions were diluted 1:5 (*v/v*) to 0.05 µg/mL for analysis by UPLC-MS/MS. Eppendorfs were weighed before and after treatment, with evaporation of the solution during treatment factored in to correct the response.

Analyte	He only			He + 0.5% O_2		
	$t_{1/2}$ (min)	*k* (min-1)	R^2	$t_{1/2}$ (min)	*k* (min-1)	R^2
MC-RR	6.3	0.110	0.999	20.4	0.034	0.959
NOD	9.3	0.074	0.998	30.8	0.023	0.978
MC-LA	3.2	0.218	0.997	6.1	0.113	0.967
MC-LF	2.0	0.342	0.998	3.5	0.199	0.997
MC-LR	4.7	0.146	0.999	11.7	0.059	0.967
MC-LY	3.8	0.183	0.997	6.5	0.106	0.983
MC-YR	8.7	0.080	0.994	20.8	0.033	0.982
ATX-A	~ 91215	~ 7.6 e-006	0.951			
CYN	~ 89780	~ 7.7 e-006	0.970			
DA	2.7	0.2552	0.899			

Table 1. Comparison of rate (k) and half-life ($t_{1/2}$) of ACPT on the toxins using helium only and a helium/oxygen admixture.

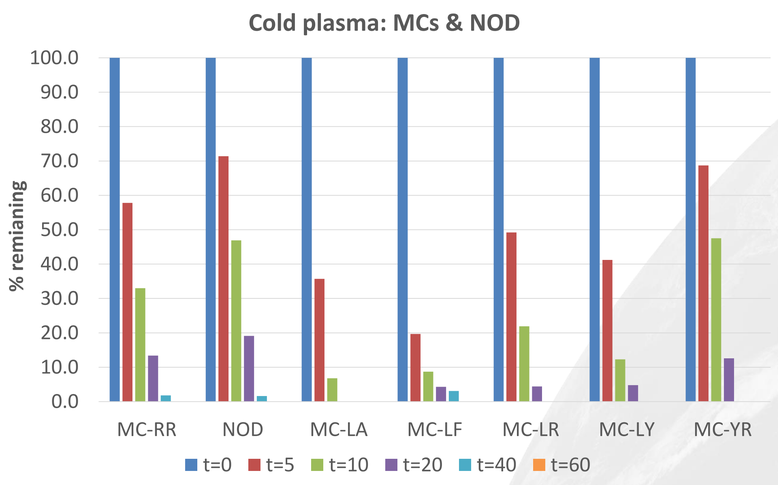

Figure 2. Results of ACPT on the MCs and NOD showing percentage of each remaining at each time point using helium gas only.

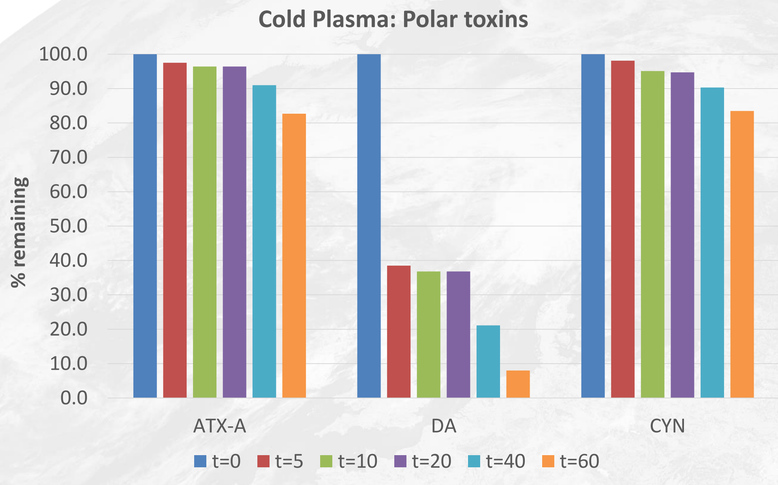

Figure 3. Results of ACPT on CYN, ATX & DA showing percentage of each remaining at each time point using helium gas only.

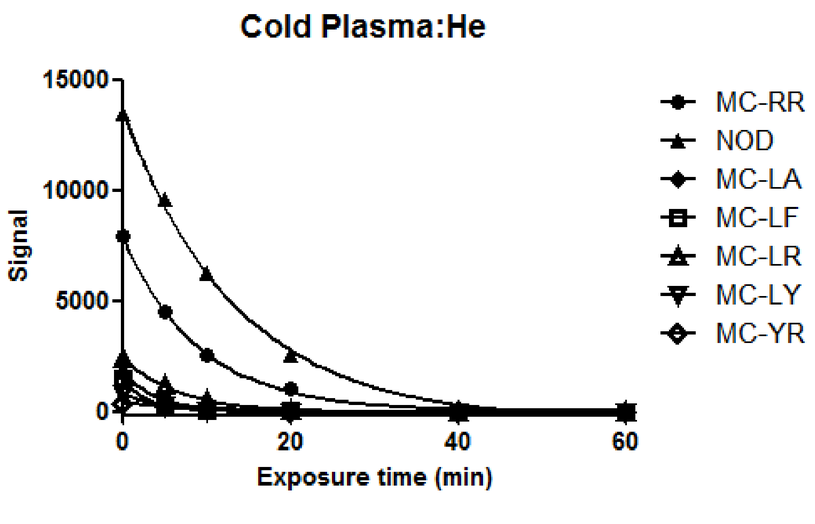

Figure 4. Degradation rate (*k*) in the first-order reaction kinetics for the MC congeners using ACPT with helium gas only.

RESULTS & CONCLUSIONS

- MCs and NOD all degraded after 60 min exposure time with helium gas only, as shown in Figures 2 and 4.
- ATX-A and CYN only showing slight degradation as indicated in Figure 3, with > 85% remaining after 60 min.
- Hydrophobic MC congeners such as -LA, -LF and to a certain extent -LY, appear the most susceptible to ACPT, having higher degradation rates (*k*) and lower half-lives ($t_{1/2}$).
- Efficacy of ACPT not enhanced by introduction of molecular oxygen into gas admixture as shown in Table 1.
    - Keeps cost of treatment lower.
- MCs containing an indole ring in the main cyclic body such as MCs -LW, -WR degrade rapidly (data not shown).
- Results indicate this technique may be of use to water treatment facilities to remove the majority of cyanotoxins under these condition:
    - Dependant on cyanobacterial producers and cyanotoxins present.

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