Development of an immunoassay for near real-time detection of particulate microcystins in Lake Erie

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Introduction

Detecting and monitoring harmful algal bloom (HAB) development and toxicity are of growing importance nationally and globally, especially for freshwater systems that supply drinking water to municipalities. As highlighted by the recent 'do not drink' advisory issued for roughly 500,000 Toledo, OH USA residents in August 2014, it is essential that we continue to advance our detection capabilities for microcystins and other cyanotoxins in drinking water sources.

The Environmental Sample Processor (ESP), pictured in figure 1, is an autonomous, in-water instrument designed to assess concentrations of potentially toxic harmful algal bloom species and the toxins they produce in near real-time. Although the ESP has been deployed numerous times in marine coastal waters, this technology has not been utilized in freshwater systems to monitor potentially toxic cyanobacteria and their toxins. In a collaborative effort to bring the *in situ* capabilities of ESP instruments to the Laurentian Great Lakes, development of an efficient extraction method and immunoassay for microcystins was initiated.

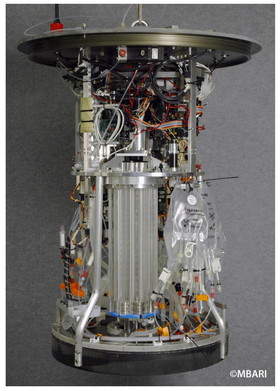

Figure 1: Image of the 2G ESP core.

Microcystin cELISA Development

Methods

Microcystin detection on board the ESP adopts a competitive Enzyme-Linked ImmunoSorbent Assay (cELISA) format. In its current configuration, the assay employs a mouse monoclonal antibody (5C4; Devlin et al., 2014) produced against MC-LR with the following cross-reactivity profile: MC-LR, 100%, MC-RR, 108%; MC-YR, 68%; MC-LA, 69%; MC-LW, 71%; MC-LF, 68%; NOD, 94%. Briefly, this antibody competes with sample or standard for binding by MC-LR-protein conjugate printed on a membrane-based array. Antibody binding to the array is visualized using a secondary antibody-HRP conjugate in combination with a substrate generating a chemiluminescent signal that is imaged by a CCD camera. Mouse IgG control features are also printed on the array and serve as a QC check for the assay chemistry as well as autoexposure metering for images. Calibration of the assay is performed using a MC-LR certified reference material (CRMP-NRC Canada).

Results and Discussion

A preliminary calibration curve for the MC cELISA is shown in Figure 2. The EC_{50} for the assay (i.e., MC concentration yielding a half maximal response) was calculated to be 40.8 ng/mL, with an estimated lower limit of detection (LOD) near 10 ng/mL. Optimization of the assay is currently underway via manipulation of several variables, including primary and secondary antibody dilutions, amount of toxin-protein conjugate per array feature, etc. It is expected that the lower LOD 'in-assay' can be decreased to about 1 ng/mL (ppb) or below, with fine-tuning of these assay variables. For detection of toxin occurring in natural waters, the actual lower LOD for the MC cELISA conducted on the ESP will be a function of sample volume and sub-ppb values should be attainable.

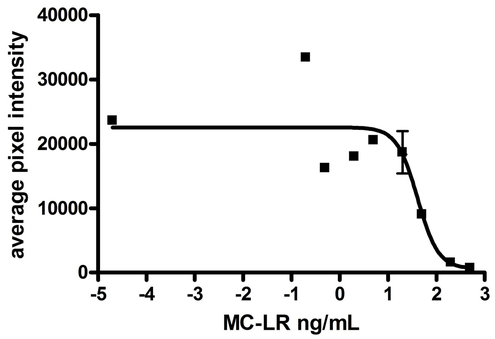

Figure 2: Preliminary calibration curve for MC cELISA conducted on an ESP benchtop mimic. Assay data were fitted using a 4-PL equation with variable slope (Prism v4; GraphPad Software, Inc.). The EC_{50} was calculated to be 40.8 ng/mL.

Testing of an ADDA-specific monoclonal antibody will be performed, as a likely replacement for the 5C4 antibody currently being used. Given the wide-spread adoption of ADDA-specific commercial ELISA kits for MC screening by a wide range of Federal, state, and private groups, incorporating an ADDA-specific antibody into the ESP MC cELISA is anticipated to provide results more directly comparable with those generated by commercial ELISA kits. Such comparability will be essential to the ESP's ability to provide relevant, real-time early warning data to stakeholders.

Development of Microcystin Extraction Procedure

Methods

While the Abraxis QuikLyse™ extraction method is widely accepted as a 'gold standard' protocol, its temperature (freeze/thaw) and mechanical (shaking) demands limit its feasibility for use on the ESP. The ideal extraction procedure will maximize recovery rate (optimally >90%) while accommodating the constraints of the ESP which can apply only heat, pressure, and/or chemical solvents.

Using laboratory cultures of microcystin-producing *Microcystis,* isolated from Lake Erie, microcystin was extracted using the Abraxis QuikLyse™ method, two modified versions of the Abraxis QuikLyse™ method, and series of 6 methanol/0.01% Tween 20 extractions using 5%, 10%, 20%, 30%, 40%, and 50% methanol respectively (Figure 3) . Following extraction, microcystin was quantified using the Abraxis Microcystin/Nodularins (ADDA) ELISA kit.

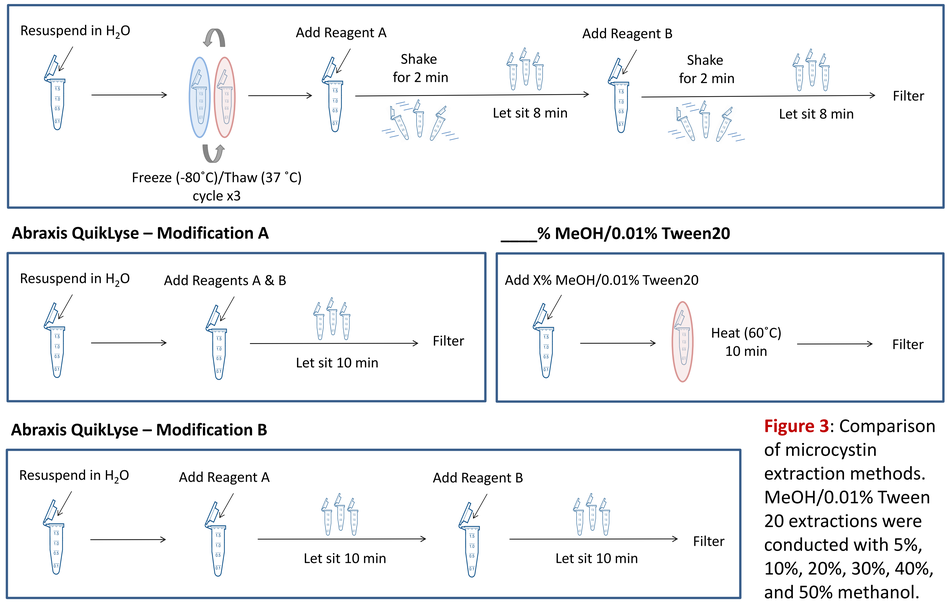

Figure 3: Comparison of microcystin extraction methods. MeOH/0.01% Tween 20 extractions were conducted with 5%, 10%, 20%, 30%, 40%, and 50% methanol.

Results and Discussion

The microcystin extraction procedures showing the highest recoveries were the Abraxis QuikLyse™ modification A method and the 5% MeOH/0.01% Tween 20 method.

The Abraxis QuikLyse™ modification A method will require the ESP to deliver two separate reagents for sample extraction, a feature which is not currently configured on the ESP but can be accommodated. Although the Abraxis QuikLyse™ modification B procedure would be easily adapted within the functional constraints of the ESP, this method yielded a very poor recovery rate (Table 1) and was therefore eliminated as a possibility.

Table 1: Average percent recovery of extraction methods relative to the Abraxis QuikLyse™ method.

Scenario	Average % recovery
Abraxis QuikLyse™ , modification A	13
Abraxis QuikLyse™, modification B	103
5% MeOH/0.01% Tween 20	101
20% MeOH/0.01% Tween 20	113
50% MeOH/0.01% Tween 20	97

A good recovery rate was obtained using >5%MeOH (Table 1), however, if dilution to 5% MeOH following extraction is required to avoid interference with the microcystin immunoassay, use of >5% MeOH for extractions will be problematic. Nonetheless, high (50%) levels of MeOH are currently used to extract other algal toxins (domoic acid, saxitoxin) on the ESP with detection by immunoassay. Safe exposure of any selected reagents to elevated temperatures and pressures on the ESP also must be considered.

Summary & Next Steps

- The ideal extraction procedure will maximize recovery rate wile accommodating the constraints of the ESP – applying only heat, pressure, and/or chemical solvents.
- A competitive Enzyme-Linked ImmunoSorbent Assay (cELISA) will be used on the ESP for microcystin detection

The exploration of microcystin extraction procedures will continue and will be expanded to include two additional methods: Milli-Q water/0.01% Tween 20 and ZyGem reagent, both conducted over a series of heat treatments.

Following further optimization of the microcystin cELISA and selection of a preliminary extraction procedure, evaluation of the extraction solvent and sample matrix effects on the assay will be conducted.

Evaluation of the extraction procedure and immunoassay will need to be conducted on the ESP once the instrument construction is complete. Extraction efficiencies can change between benchtop and on ESP analyzes using the same protocol.

A summer 2016 deployment of the ESP in western Lake Erie (Figure 4) is anticipated for near real-time detection of particulate microcystins.

Figure 4: MODIS satellite image of Lake Erie during a bloom in the western basin in September 2013.

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