



# Water Treatment Optimization for Cyanotoxins

Version 1.0



## Disclaimer

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The intent of this document is to provide treatment considerations for water treatment plant managers, supervisors, and operators faced with harmful algal blooms in their source water. The approaches presented in this document are not intended to be mandates or directive to any entities.

The science on water treatment optimization for cyanotoxins is still evolving. The U.S. Environmental Protection Agency (U.S. EPA) will update this document as more research and information become available. EPA certainly welcomes comments and feedback on the content of this document.

The focus of this document is on water treatment optimization for cyanotoxins. Future versions of this document may include additional information or resources on source water protection as a strategy for preventing cyanotoxins in drinking water sources.

For purposes of this document, the term "Harmful Algal Blooms" (HABs) refers to cyanobacteria (sometimes called "blue-green algae") blooms with the potential of producing cyanotoxins. U.S. EPA recognizes that alternative descriptors (e.g., Harmful Cyanobacteria Blooms [HCBs], cyanoHABs, cyanobacteria HABs [CHABs]) have been used elsewhere but considers "HABs" a widely used and recognized term. U.S. EPA may substitute an alternative term in future updates to this document if consensus builds around such an alternative.

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## Abbreviations

ADDA	3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid. An amino acid that is part of the microcystin molecule and is common to a majority of microcystin congeners.
AMWA	Association of Metropolitan Water Agencies
ASDWA	Association of State Drinking Water Agencies
AWOP	Area-Wide Optimization Program
AWWA	American Water Works Association
CCP	Composite Correction Program
CO <sub>2</sub>	Carbon dioxide
DBP	Disinfection by-product
DNA	Deoxyribonucleic acid
EBCT	Empty-bed contact time
ELISA	Enzyme-linked immunosorbent assay
GAC	Granular activated carbon
HAA5	Haloacetic acids (5 regulated compounds)
HAB	Harmful algal bloom
HESD	Health effects support document
IFE	Individual filter effluent
LC/MS/MS	Liquid chromatography / tandem mass spectrometry
MF	Microfiltration
MRDL	Maximum residual disinfectant level
NAWC	National Association of Water Companies
NF	Nanofiltration
NOM	Natural organic matter
NTU	Nephelometric turbidity unit. A measure of turbidity.
PAC	Powdered activated carbon
PWS	Public water system
RO	Reverse osmosis
RSSCT	Rapid small-scale column test
TOC	Total organic carbon
TTHM	Total trihalomethane
T&O	Taste and odor
UF	Ultrafiltration
USEPA	United States Environmental Protection Agency
UV	Ultraviolet (light), typically used in water treatment as an oxidant
WRF	Water Research Foundation

## Section 1: Background and Introduction

Increasing occurrence and detection of harmful algal blooms (HABs), sometimes referred to as blue-green algae or cyanobacteria blooms, in drinking water sources pose a variety of challenges to water treatment plant managers and operators. In addition to taste and odor issues that may be associated with algal blooms, HABs sometimes produce cyanotoxins, to which human exposure can result in a host of adverse health effects, including gastroenteritis, and damage to the liver, kidneys, or nervous system (U.S. Environmental Protection Agency, 2015a-f).

In June 2015, the U.S. Environmental Protection Agency released Health Advisories for two specific cyanotoxins – total microcystins and cylindrospermopsin – and Health Effects Support Documents (HESDs) for three specific cyanotoxins – total microcystins, cylindrospermopsin, and anatoxin-a. At the time of release, there was not sufficient information to develop a Health Advisory for anatoxin-a (U.S. EPA, 2015a,b,d-f). The Health Advisories include information on health effects, analytical methods and water treatment. The HESDs provide a comprehensive review of published literature on physical and chemical properties, environmental fate, known occurrence information, and health effects. Additionally, EPA released a supporting document titled “Recommendations for Public Water Systems to Prepare for and Respond to Cyanotoxins in Drinking Water” (U.S. EPA, 2015c). That “Recommendations” document is intended to assist public drinking water systems (PWSs) in managing the risks from cyanotoxins in drinking water. It includes information for evaluating source waters for vulnerability to contamination by cyanotoxins and describes a framework for managing risks to cyanotoxins that PWSs can consider in determining their risk management efforts. Appendix E of the “Recommendations” document also contains information on long-term mitigation strategies and treatment options (U.S. EPA, 2015c).

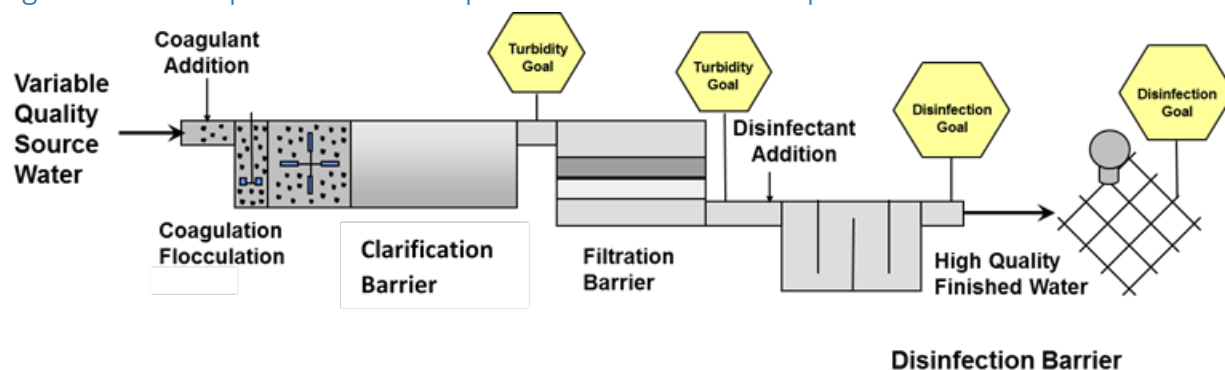
The American Water Works Association (AWWA) and the Water Research Foundation (WRF) have also released “A Water Utility Manager’s Guide to Cyanotoxins.” That guide provides a brief overview of current knowledge surrounding common questions utility managers may have, to help them better prepare for cyanotoxins and to respond when cyanotoxins cause water quality problems (AWWA, 2015). WRF, in conjunction with Water Research Australia, released a report titled “Optimizing Conventional Treatment for the Removal of Cyanobacteria and Toxins” that provides detailed guidance to water utilities on the optimization of conventional treatment practices (including coagulation, clarification, and filtration) for the removal of cyanobacteria and their toxins while meeting all other water quality goals associated with drinking water production (Newcombe, et al., 2015).

The purpose of this document is to assist PWS managers and operators (as well as technical assistance providers working with PWS personnel) with preparing for, and responding to, the treatment challenges that often arise during HAB events and introduce principles that can be used to achieve optimization goals using a compilation of published approaches and strategies. This document complements the USEPA Health Advisories for total microcystins and cylindrospermopsin and the “Recommendations” document.

For the purposes of this document, treatment optimization is defined as achieving the best performance possible from each unit process in a water treatment plant by applying process control techniques and

problem-solving skills (e.g., priority setting, tailored studies, and data trending), while continuously assessing unit process performance relative to pre-established goals. In support of this, water plant operators should consider routinely monitoring water quality from each unit process, as well as raw and finished water quality. Operators can analyze trends from the monitoring data to assess system performance and the impact of process controls relative to optimization goals. As presented in EPA’s Composite Correction Program (CCP) (U.S. EPA, 2004), the primary approach to optimization for protection from waterborne disease and removal of contaminants is the “multiple-barrier” concept, which currently includes treatment and distribution system barriers. This is shown below in Figure 1-1. A commonly used performance indicator for waterborne pathogens is particulate removal as measured by turbidity. The CCP has established turbidity goals for sedimentation and filtration.

Figure 1-1. Multiple barrier concept for water treatment optimization



EPA’s CCP handbook presents the concept of a “capable plant” with respect to microbial (turbidity-based) water treatment optimization (U.S. EPA, 2004). A water treatment plant capable of optimization not only relies on good design, but also has supportive administration/management and is well operated and maintained. In this vein, the CCP outlines a series of performance-limiting factors in each of these categories (administration, design, operation, and maintenance), which can impact a system’s ability to remove pathogens<sup>1</sup>.

Specific, generally accepted in-plant cyanobacteria and cyanotoxin targets (i.e. goals) have yet to be established to formally define optimization for these parameters. This document represents a step toward that objective. Jar testing, for example, is commonly used to optimize particulate removal (which could include cyanobacteria cells) in water treatment plants. However, current research suggests that under certain conditions, turbidity, which is a common optimization parameter for jar testing, may not be a good indicator of cyanobacteria cell or toxin removal (Newcombe, et al., 2015), and that alternative water quality parameters, such as pigments chlorophyll-*a* and phycocyanin (fluorescence), UV254

<sup>1</sup> The USEPA’s Area-Wide Optimization Program (AWOP) and the Partnership for Safe Water are two programs that promote drinking water optimization. The Partnership for Safe Water is an alliance of the American Water Works Association (AWWA), Association of Metropolitan Water Agencies (AMWA), Association of State Drinking Water Administrators (ASDWA), National Association of Water Companies (NAWC), USEPA, and the Water Research Foundation (WRF).

[Additional information about the AWOP](https://www.epa.gov/dwstandardsregulations/optimization-program-drinking-water-systems) can be found at <https://www.epa.gov/dwstandardsregulations/optimization-program-drinking-water-systems>



(organics), and surface charge, should be investigated for optimizing each water treatment unit process for cyanobacteria cell removal (e.g., through jar testing).

Cyanotoxins present some unique challenges for water treatment plants, which may include:

- Chemical and biological parameters can vary widely in source water, both over time and by location.
- The presence of cells does not necessarily mean that toxins are present at any given time. Toxins can also be present even when cell concentrations are low. High toxin concentrations can also persist after blooms/cells are no longer in the source water.
- Cyanotoxins can be located within the intact cyanobacteria cell (termed “intracellular” or “cell-bound”) or outside the cell within the water matrix (termed “extracellular” or “dissolved”). Some water treatment approaches, including the application of particular oxidants/disinfectants, can release the toxins from the cyanobacteria cells, thereby increasing the extracellular toxin concentration. Therefore, the choice and location of oxidant application can be challenging.

Responding to these challenges requires balancing multiple, and sometimes competing, treatment objectives. The following sections discuss monitoring and treatment optimization for removing cyanotoxins while attempting to address potentially competing treatment objectives.

## Section 2: Understanding source water to anticipate treatment needs

### 2.1 Ambient source water conditions that favor cyanobacteria proliferation

Cyanobacteria are often part of a healthy aquatic ecosystem and exist in balance with other aquatic organisms. HABs occur when certain water quality, hydrologic, environmental, climatic, and atmospheric conditions favor cyanobacteria proliferation. Availability of nutrients, particularly nitrogen and phosphorous, tends to impact cyanobacteria growth in most water bodies. Therefore, when nutrient levels increase, the mass of cyanobacteria usually increases. Temperature and light intensity affect cyanobacteria proliferation as well. Warmer water temperatures, typically 25°C (77°F) or greater, favor growth of some cyanobacteria. *Microcystis* growth is usually limited below 15°C (59°F) (Robarts and Zohary, 1987), but other cyanobacteria, such as *Planktothrix* and *Cylindrospermopsis*, can survive in colder temperatures, even under ice, either as akinetes or in a vegetative state (Dokulil, 2016; Holland & Walsby, 2008). Because cyanobacteria are photosynthetic, they favor long periods of sunlight. Wind patterns can also influence the location of the bloom and concentration of cyanobacteria cells (and therefore potential cyanotoxin concentration) on a specific water body. Warmer, calmer, shallow bays are conducive for cyanobacteria bloom formation, as are shallow inlets that receive high nutrient loads. Wind patterns have the potential to concentrate blooms into scums, which can represent a thousand-fold to million-fold concentration of cyanobacteria cell populations (Chorus & Bartram, 1999). This is especially concerning if the wind direction and scum formation occur in the vicinity of a PWS intake. Table 2-1 summarizes water quality conditions favorable for cyanobacteria proliferation:

Table 2-1. Water quality conditions favorable for cyanobacteria proliferation

Source Water Condition:	When to take notice:
<b>Excessive nutrients</b>	Nitrogen and/or phosphorous are the primary nutrients of concern for cyanobacteria. Elevated nitrogen and/or phosphorous levels can lead to cyanobacteria proliferation. Different water bodies will have different levels of nutrients that can favor cyanobacteria proliferation.
<b>Quiescence</b>	Calm, stagnant waters (i.e., low flow or slope in rivers; low turnover or wind conditions in lakes/reservoirs; etc.).

Weather Conditions	When to take notice:
<b>Water temperature</b>	Water temperatures above 25°C (or lower for some cyanobacteria species)
<b>Light intensity and rainfall</b>	Rainfall followed by prolonged periods of sunlight and dry conditions. Rain washes nutrients into the water body and subsequent sunny and dry conditions can lead to cyanobacteria proliferation.
<b>Wind patterns</b>	Wind conditions that concentrate surface blooms in warm, shallow parts of a water body in the vicinity of nutrient sources. Strong winds can also mix surface blooms downward toward intake depths.

Competition between algal species also occurs as conditions change. Each algal species has its optimal conditions, such as light, water temperature, pH, nutrients, etc. that control their proliferation in a given water body (AWWA, 2010). As noted above, not all algal blooms consist predominantly of cyanobacteria, and not all cyanobacteria blooms produce cyanotoxins; therefore, water quality monitoring is important because it can provide baseline information that will assist PWSs in understanding critical factors in source water that contribute to cyanobacteria blooms.

## 2.2 Bloom identification, confirmation, and quantification in source water

A good understanding of the vulnerability and historical cyanobacteria and cyanotoxin levels in a water treatment plant’s source water allows utilities to take proactive and preventative approaches to water treatment in the plant. In support of this, adequate time for sampling and analysis should be built into a proactive monitoring approach, with the understanding that different cyanotoxin methods require different amounts of time to generate results.

### 2.2.1 Indirect cyanobacteria screening methods

Often, the most obvious sign of a HAB is simply visual. Operators are encouraged to visually inspect their source water regularly, especially in the vicinity of intakes. There are several inexpensive indicators that treatment plant operators can proactively monitor in their source water that may suggest action to identify elevated cyanobacteria biomass levels and potential cyanobacteria problems. This entails

routine monitoring and trending of the data to establish baseline levels for their source water and identify changes in these levels that may merit a monitoring/treatment response. Detecting significant deviations from normal, or baseline levels, of these indicators would alert the operator to begin direct screening for cyanobacteria and analyses for cyanotoxins; however, these indicators do not take the place of quantitative, confirmatory measurements. Table 2-2 summarizes some source water measurements that could be utilized as indicators of potential cyanobacteria problems. Measuring a combination of these indicator parameters as part of the HAB screening process best allows one to assess source water.

Table 2-2. Source water quality indicators

Parameter	Description
<b>Pigments: Chlorophyll-<i>a</i> and/or Phycocyanin</b>	Extractive pigment measurements (as differentiated from probes or flow-through instruments) include EPA Method 445.0 for chlorophyll- <i>a</i> (U.S. EPA, 1997), which can be run at a water plant. Another site-specific parameter is phycocyanin. Phycocyanin is a pigment unique to cyanobacteria, and elevated levels can indicate cyanobacteria proliferation (Brient, et al., 2008; AWWA, 2010; Kasinak et al., 2015) and differentiate from other types of algae. At this time there is not a published EPA method for phycocyanin. There are, however, numerous commercial in-vivo <sup>2</sup> methods for measuring phycocyanin and chlorophyll- <i>a</i> with probes or flow-through instruments. These can be placed on a buoy, inline at a raw water intake, in a pump wet well, etc.
<b>Turbidity</b>	There are limitations on using turbidity as an indicator for cyanobacteria cell concentration, especially if raw water is less than 10 NTU (Newcombe, et al., 2015); however, unexplained increases (e.g., with no recent rainfall) may be related to increases in cyanobacteria biomass.
<b>Secchi depth</b>	This is a simple, inexpensive test for source water. Decreases in Secchi depth may indicate increased cyanobacteria concentration, although it has limitations similar to those with turbidity.
<b>Diurnal pH changes or increases in pH associated with a bloom</b>	Diurnal changes in source water pH (increasing during the day, decreases at night) or prolonged increases in pH could indicate the presence, or proliferation, of cyanobacteria. This is due to the cyanobacteria's photosynthesis (light availability

<sup>2</sup> In-vivo fluorescence methods that are typically used with probes or flow-through instruments are based on illuminating the sample with light at phycocyanin's excitation wavelength and then reading the response at the emission wavelength.

Parameter	Description
	and CO <sub>2</sub> absorption) during the day and cell respiration at night. (Usui and Kasubuchi, 2011; Usui, et al., 2003).
<b>Taste &amp; odor (T&amp;O) compounds (MIB, geosmin, β-cyclocitral)</b>	T&O compounds may coexist with some cyanobacteria blooms, and depending on the species and type of T&O compound, could be indicators of cyanobacteria presence <sup>3</sup> . However, it is possible to have T&O without cyanotoxins and vice versa.
<b>Temperature, nutrients</b>	Elevated temperatures and nutrient (nitrogen and phosphorus) concentrations are more conducive to producing cyanobacteria blooms.
<b>Natural organic matter (NOM)</b>	Increases in NOM in source water, with no recent rainfall, could be indicative of a cyanobacteria issue.

Operators may also notice changes within the treatment plant that could be indicative of cyanobacteria in the source water. These could include:

- An increase in color (most likely green) observed visually in the raw water or elsewhere within the treatment plant (e.g., in clarifiers or dissolved air flotation (DAF) and filter surface scums).
- An increase in treatment difficulties, for example, decreased filter run times, increased chemical needs/usage, difficulty in maintaining a finished water residual or meeting turbidity goals.
- Observation of seasonal shifts in treatment. Consideration of these seasonal changes could help shape HAB monitoring programs or HAB treatment planning.

### 2.2.2 Direct cyanobacteria measurement methods

Direct measurements of cyanobacteria proliferation in source water is ideal, but there are limited options available. Species identification is an important factor in understanding the type of cyanotoxins potentially present, which is helpful information in determining the appropriate cyanotoxin analysis. Cyanobacteria cell counting and identification can be accomplished by analysts trained in microscopy and algal identification. Assistance and confirmation may be necessary from a trained phycologist, or other appropriately-trained individual. DNA technology for identifying cyanobacteria and cyanotoxin production capacity in source water is an emerging tool. Research suggests that the microcystins-producing gene cluster is rarely present without the toxin being synthesized by the cell. Therefore, gene sequencing, such as that accomplished through polymerase chain reaction (PCR), could be an option for monitoring. Commercial molecular assays that utilize certified reference materials are available to help standardize this method. Because molecular techniques require specialized equipment and training to run the method and interpret the results, their use is probably limited to utilities with access to more

<sup>3</sup> Note that *Microcystis* does not produce the most common T&O compounds, methylisoborneol (MIB) or geosmin. This genus usually produces β-cyclocitral, dimethyl trisulfide (DMT), diisopropyl disulfide (DID), and/or diisopropyl trisulfide (DIT). β-cyclocitral is a T&O compound produced during cell damage or death (AWWA, 2010), which could be indicative of *Microcystis* cell lysis (and thus, potential toxin release).

sophisticated labs. For more information on cyanobacteria identification and differentiation from algae, see Rosen and St. Amand (USGS), 2015 or AWWA M57, 2010.

## 2.3 Cyanotoxin measurement

The monitoring methods mentioned above help alert operators to the presence of cyanobacteria and potential presence of cyanotoxins in their source water. These indicators may signal the need for more quantitative, confirmatory measurements, cyanotoxin analyses.

Qualitative screening tests, such as commercially-available immunochromatographic strip tests for microcystins<sup>4</sup>, cylindrospermopsin, and anatoxin-a, can be useful to determine the presence or absence of cyanotoxins in water samples and alert utilities of the need for further, more quantitative cyanotoxin analysis. These analyses can take approximately one hour, or more or less depending on the method, sample preparation requirements, and individual lab practitioner.

When conducting quantitative cyanotoxin analyses, the type and form of cyanotoxins are important for a water treatment plant operator to determine, as treatment approaches can differ significantly based on which cyanotoxin is present and whether or not the cyanotoxins are primarily intracellular (located inside the cyanobacteria cell) or extracellular (located outside the cyanobacteria cell). Regardless of the analytical method, sample preparation determines whether the measured cyanotoxins are intracellular or extracellular. Typically, a sample is split into two. One fraction is filtered or centrifuged before analysis. The filtrate/supernatant is used for the analysis, yielding a measurement of extracellular cyanotoxin concentration. The other sample fraction is lysed (e.g., freeze-thaw, sonication techniques) before analysis – releasing the cyanotoxins from the cells. The subsequent analysis will then yield the total cyanotoxin concentration. The intracellular fraction is then calculated by the difference, such that:

$$\text{[Total concentration]} - \text{[Extracellular concentration]} = \text{[Intracellular concentration]}$$

The following subsections describe some of the more common analytical methods for evaluating cyanotoxins in water. The description is not all-inclusive, and the body of available analytical methods continues to grow. When considering various methods, one should verify that the detection limit is appropriate for finished water characterization (e.g., the Health Advisory levels for children less than six years old of 0.3 µg/L for total microcystins and 0.7 µg/L for cylindrospermopsin). As with any analytical method, it is important to follow the established sample collection, preservation, storage, and preparation steps (Kamp et al., 2016). For example, it is important to quench<sup>5</sup> finished water samples during collection if exposed to oxidants, protect samples from sunlight, and chill samples at a temperature according to the method prior to analysis (Ohio EPA, 2015; USEPA, 2015g,h; Kamp et al., 2016).

### 2.3.1 Enzyme-linked immunosorbent assay (ELISA)

Enzyme-linked immunosorbent assay (ELISA) kits are commercially available for measurement of microcystins and cylindrospermopsin, saxitoxin, and anatoxin-a. These assays utilize antigen/antibody interactions to identify and quantify chemical contaminants and can be either “competitive”, where color response is inversely proportional to the toxin concentration, or “non-competitive”, where color

<sup>4</sup> Options are available for microcystins for both source (with a lysing step) and finished water.

<sup>5</sup> For example, with sodium thiosulfate, depending on the method requirements, type of oxidant present, and analyte.

response is directly proportional to the toxin concentration (AWWA, 2010). Because the EPA Health Advisory for microcystins applies to “total” microcystins, as opposed to a specific microcystin congener(s), operators will generally want to use a method that addresses microcystins broadly. The ADDA-ELISA test kits detect the standard 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid (ADDA) moiety in the microcystin molecule, which is a unique amino acid within the molecule that is common to a majority of microcystin congeners and a related cyanotoxin, nodularin (Fischer et al., 2001). Thus, this competitive assay technique is generally believed to provide a good representation of total microcystins. USEPA has published EPA Method 546 for determination of total microcystins and nodularin by ADDA-ELISA (USEPA, 2016). In addition, the Ohio EPA has posted an “Analytical Methodology” for measuring microcystins with the commercially-available ADDA-ELISA technique to its website as a resource for water treatment plant operators (Ohio EPA, 2015). To achieve representative, reliable results, it is important to have a consistent approach and adhere to the terms of the method. EPA Method 546 establishes specific sample collection, preservation, storage, quality control, calibration, and data analysis criteria in order to ensure accuracy and precision of the method.

Table 2-3. Advantages and disadvantages of ELISA for cyanotoxin monitoring

Strengths	Limitations
<ul style="list-style-type: none"> <li>• Method is relatively easy to learn and use.</li> <li>• Relatively inexpensive (as low as \$11 per sample [as of 2015]<sup>6</sup>, assuming full plates are run).</li> <li>• Can be run relatively quickly compared to LC/MS/MS techniques (discussed further below) – typically about 4 hours, including sample preparation.</li> <li>• Numerous manufacturers of ELISA kits.</li> <li>• The ADDA-based measurements are congener-independent (i.e., the ADDA structure is common to a majority of microcystin congeners), therefore providing an indication of “total microcystins”.</li> </ul>	<ul style="list-style-type: none"> <li>• Since the method involves working with small volumes, it requires good micropipette skills and an 8-channel pipette. Good laboratory technique is important to achieve consistent results.</li> <li>• The calibration curve is non-linear (four-parameter logistic equation) and accuracy may be questionable at concentration ranges above or below the linear portion of the calibration curve (see EPA Method 546 [USEPA, 2016] for a discussion on the EC<sub>50</sub> and establishing a minimum reporting level [MRL] and calibration curve).<sup>7</sup></li> <li>• Calibration is based on a microcystin-LR standard and other microcystin congeners have exhibited variable cross-reactivity relative to microcystin-LR. This may introduce error into the total microcystins result.</li> </ul>

<sup>6</sup> This is a minimum cost estimate and assumes full use of the plate (all wells) with the minimum dedicated to calibration/control. Assumes \$440 for the ELISA ADDA kit, 96 wells with 16 wells dedicated to calibration/control and 80 wells for samples. Assumes duplicate wells per sample, therefore there is room for 40 unknowns, which equals \$11 per sample. The upper bound of this estimate, that is, running only one sample per plate, would be the price of the kit itself (i.e., \$440 per sample), assuming the minimum 16 wells are dedicated to calibration/control. Therefore, the more samples run on a given plate (up to 40), the less expensive each analysis becomes.

<sup>7</sup> As stated in EPA Method 546, the “EC<sub>50</sub> is the concentration of microcystin that yields an absorbance halfway between the bottom plateau of the calibration curve and the top plateau. The EC<sub>50</sub> is the concentration at the inflection point (of the calibration curve) and is in the center of the most reliable measurement range...”.

Strengths	Limitations
	<ul style="list-style-type: none"> <li>• Does not yield congener-specific information.</li> <li>• Since the different manufacturers generally use proprietary antibodies in the assay, variations in results between manufacturers may be expected.</li> <li>• Since LC/MS/MS methods for microcystins focus on a limited number of congeners, and since standards are only available for a limited number of microcystin congeners, one cannot truly “confirm” ELISA-based microcystins results with LC/MS/MS methods.</li> </ul>

### 2.3.2 Liquid chromatography with tandem mass spectrometry (LC/MS/MS)

Liquid chromatography is a separation technique that allows for mass analysis by mass spectrometry. Most water utilities do not have LC/MS/MS capabilities in-house and utilize contract laboratories for this purpose. USEPA has published two cyanotoxin-related analytical methods for LC/MS/MS – EPA Method 544, which measures six microcystin congeners (MC-LA, MC-LF, MC-LR, MC-LY, MC-RR, and MC-YR) and nodularin (U.S. EPA, 2015h), and EPA Method 545, which measures anatoxin-a and cylindrospermopsin (U.S. EPA, 2015g). Others have developed LC/MS/MS methods as well. Note that EPA Methods 544 and 545 were developed for analysis of finished drinking water samples. Work is underway to expand the scope of EPA Methods 544 and 545 to ambient (source) water samples.

While there can be value in using LC/MS/MS techniques to understand the occurrence of particular microcystin congeners, as of the time this document was written, they cannot practically be used to “confirm” ELISA results. LC/MS/MS methods for microcystins currently focus on a limited number of specific microcystin congeners, whereas ELISA methods measure microcystins more broadly.

Work is underway to evaluate an LC/MS/MS technique that is based on measuring 3-methoxy-2-methyl-4-phenylbutyric acid (“MMPB”). MMPB is an oxidation product of microcystins (e.g., produced via oxidative cleavage) and has the potential to serve as a measure of microcystins, broadly. Foss et al. found that “(w)hen summarizing the total microcystins detected in raw samples..., the MMPB method accounted for an average of 99% of the microcystins detected using ELISA, while individual variant analysis of 13 congeners using LC/MS/MS accounted for 81%” (Foss and Auel, 2015). The LC/MS/MS MMPB technique has proven valuable for confirming ELISA results from raw water samples, but may have some limitations with finished water analysis due to potential for detection of microcystins oxidation byproducts.

LC/MS/MS can reliably confirm cylindrospermopsin or anatoxin-a ELISA results.

Table 2-4. Advantages and Disadvantages of LC/MS/MS for cyanotoxin monitoring

Strengths	Limitations
<ul style="list-style-type: none"> <li>• Congener-specific measurement</li> </ul>	<ul style="list-style-type: none"> <li>• Only measures compounds for which an analytical standard exists.</li> </ul>

Strengths	Limitations
<ul style="list-style-type: none"> <li>Precise, accurate measurement. Can measure specific microcystin congeners, cylindrospermopsin and anatoxin-a.</li> </ul>	<ul style="list-style-type: none"> <li>EPA Methods 544 and 545 are currently applicable to finished water samples only.</li> <li>More expensive than ELISA.<sup>8</sup></li> <li>Requires specialized equipment and experienced analyst.</li> <li>The LC/MS/MS results may take longer to receive, compared to ELISA analysis, if measured by an offsite laboratory.</li> </ul>

### 2.3.3 Cyanotoxin toxicity measurement

ELISA and LC/MS/MS methods are used to directly measure cyanotoxin concentrations. There are also “indirect” methods that focus on measures of toxicity, such as phosphatase inhibition assay kits. These tend to be less selective than ELISA and typically yield results presented in “microcystin-LR toxicity equivalents” (AWWA, 2010). It is unclear how applicable the toxicity equivalent is to judge a utility’s risk, since EPA’s current Health Advisory is based on cyanotoxin concentration.

### 2.4 Source water considerations and associated short-term management strategies

Source water quality management strategies are usually short-term solutions to larger nutrient loading issues. Source water management techniques may be able to temporarily treat HABs, however the underlying cause of HABs is usually nutrient loading on the water body (nitrogen and phosphorous) (NEIWPC, 2015). If screening or monitoring shows an increasing trend in cyanobacteria, cyanotoxin, or indirect indicators, response strategies for water treatment plant operators generally include exploring various treatment adjustments that can be made in order to minimize cell-bound (intracellular) cyanotoxin release and passage into finished water. Additional strategies may be needed if extracellular cyanotoxins are present. More detailed treatment information is provided in Section 3, however some near-term source water treatment strategies, which will have system-specific applicability, include:

- Continue monitoring biomass (cell identification and enumeration, or screening indicator parameters) and toxin type and concentration to determine where toxins are located within the water source (i.e., relative to the plant intake).
- Understand whether the toxins are primarily intracellular or extracellular to best direct treatment strategies.
- Monitoring water quality at various depths and changing intake levels as warranted. Often cyanobacteria cells are located at different levels in the water column. Water quality monitoring at each depth is important to help discern whether changing the intake level would avoid the bloom – and not significantly compromise other treatment objectives (e.g., manganese removal, disinfection byproduct control, corrosion control).

<sup>8</sup> Costs for LC/MS/MS are dependent on several factors including whether the utility has access to LC/MS/MS instrumentation and the cost of commercial lab analysis (which varies). For resources to assess cost and laboratory availability, see [USEPA’s CyanoHAB website](https://www.epa.gov/nutrient-policy-data/state-resources) (https://www.epa.gov/nutrient-policy-data/state-resources). The New England Interstate Water Pollution Control Commission (NEIWPC) also has produced a list of laboratories that provide cyanobacteria and cyanotoxin services, including costs as of March 2016.



- Cease or limit algaecide applications to source waters in use. In some instances, algaecide can prevent full proliferation of a bloom if it is applied very early in the bloom cycle, such that the low cell count would result in low toxin levels if cells did lyse and release toxins. However, algaecide application during a full cyanotoxin-producing bloom is generally discouraged as it can stress or lyse cells resulting in high levels of cyanotoxin release. For source waters actively being used, consider sampling the bloom for cyanotoxins prior to any algaecide application to evaluate the potential for cyanotoxin release following application. Alternatively, one can temporarily discontinue use of a source water during algaecide application and sample for cyanotoxins prior to placing it back in service.
- Utilize an alternate water source (i.e., location not impacted by the bloom). If cyanotoxins are elevated to a concentration that may be difficult to treat, consider emergency interconnections with neighboring water systems, if available. Thorough source water and water quality analyses should be considered if pursuing this option.

## 2.5 Long-term strategies to prevent or mitigate cyanobacteria blooms in source water

A source watershed nutrient management plan or establishing source water nutrient goals can be an important element of a longer-term strategy to mitigate cyanobacteria blooms. Nutrients, such as nitrogen and phosphorous, are often key factors in cyanobacteria proliferation, and some states are beginning to explore watershed-wide nutrient management strategies with the goal of preventing future HABs. The New England Interstate Water Pollution Control Commission, in its Harmful Algal Bloom Control Methods Synopses document, states that “effective watershed management to reduce nutrient pollution to a waterbody is often difficult, expensive, and time consuming. Regardless, it is key to reducing the occurrence and frequency of HABs and to addressing other water quality problems associated with eutrophication” (NEIWPC, 2015). As stated in “Recommendations for Public Water Systems to Prepare for and Respond to Cyanotoxins in Drinking Water”, “Local source water assessment or protection organizations may also be leveraged to communicate key messages to the drinking water community; a few of these watershed groups can be found through the Source Water Collaborative “How to Collaborate Toolkit” (SWC, 2015a)” (U.S. EPA, 2015c). This approach is watershed-specific and would require the cooperation of multiple entities such as local governments, landowners, and nearby water utilities and wastewater plants<sup>9</sup>.

Utilities with persistent HAB problems can also explore the possibility of accessing or blending with alternate water sources that are not impacted by HABs, when needed. Multiple issues such as treatment changes, residual maintenance, corrosion control, and state review/approval would have to be addressed before implementation. Potential options include:

- Planning for alternate intake levels or locations in a HAB-impacted reservoir (i.e., identifying an area that is not impacted, or is less impacted, by the bloom). Again, water quality monitoring would be important to confirm that the alternate intake represents a superior location and that other treatment objectives are not compromised.
- Using an alternate reservoir or source of water, such as a river intake that is not impacted by HABs or groundwater. There are significant treatment and distribution system implications that must be considered if this strategy is utilized, as mentioned above.

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<sup>9</sup> Additional tools for reducing nutrients can be found through ASDWA’s website ([www.asdwa.org](http://www.asdwa.org)).

- Purchasing water from neighboring systems not impacted by HABs. This may require distribution system upgrades and may result in other secondary impacts related to distribution system water quality and hydraulics. Thorough source water and water quality analyses should be considered if pursuing this option.
- Managing source water or employing preventive treatment measures. Thorough analysis should be conducted prior to implementing these strategies to consider all potential water uses, ecological effects, design challenges, and resulting impacts on water quality. States or primacy agencies may also have regulations that may apply to some source water management / preventive treatment measures discussed here:
  - Utilities can consider the applicability of circulation (aeration or mixing). This can be accomplished using hypolimnetic bubble aeration or mechanical mixing. Cyanobacteria prefer calm, stagnant water in order to proliferate. Adding hypolimnetic aeration or mixing would disrupt the stratification and stagnation of the water body and mitigate cyanobacteria growth. As a cost-effective strategy, hypolimnetic aeration or mechanical mixing can be placed strategically close, and at the depth of, the water treatment plant intake.
  - Strategic, optimized timing/dose of algaecide application. The use of algaecides is generally discouraged during a full cyanobacteria bloom, as this risks cell lysis causing cyanotoxin release. However, as stated above in Section II.D, in some instances, algaecide can prevent full proliferation of a bloom if it is applied very early in the bloom cycle, such that the low cell count would result in low toxin levels if cells did lyse and release toxins. Long-term, if algaecides are necessary, utilities can investigate optimal doses and timing of algaecide application to prevent full bloom proliferation and minimize extracellular cyanotoxins.
  - Flocculants applied to source water can limit cyanobacteria bloom proliferation by addressing biologically-available phosphorous by binding and settling (sometimes referred to as “sequestration”). As stated previously, phosphorous is a key nutrient and cause of HABs in many water bodies. For example alum, a common coagulant used in conventional drinking water treatment, can be used for the purpose of phosphorous sequestration in source water. The phosphorous is removed either by precipitation / sedimentation or adsorption mechanisms.
  - Ultrasound/sonication. Above certain frequencies in water, ultrasound waves cause formation of microbubbles which, upon collapse, can damage cell walls. This is called “acoustic cavitation”. Low-power ultrasound/sonication systems are also available that use sound wave resonance in the water to collapse the gas vesicles in the cyanobacteria cell that are used to regulate the cell’s buoyancy, thus rendering the cell incapable of moving through the water column to locate optimal light conditions for photosynthesis. Different source waters with different cyanobacteria species will require different ultrasound frequencies for optimal results. A potential concern with ultrasound treatment for cyanobacteria is the possibility of cell disruption causing cyanotoxin release.
  - Some utilities have reported success with hexagonal tile covers that are placed on the surface of the source water, generally for small water bodies, forebays or lagoons. The tile covers can serve two purposes in mitigating cyanobacteria proliferation: (1) reducing

sunlight exposure of the water, thereby reducing photosynthetic activity; and (2) deterring birds and other waterfowl from using the water body, which could reduce this source of nutrient inputs.

- For more detailed information on source water management and preventive treatment strategies for HABs, the New England Interstate Water Pollution Control Commission (NEIWPC) has developed a Harmful Algal Bloom Control Methods Synopsis document (NEIWPC, 2015).

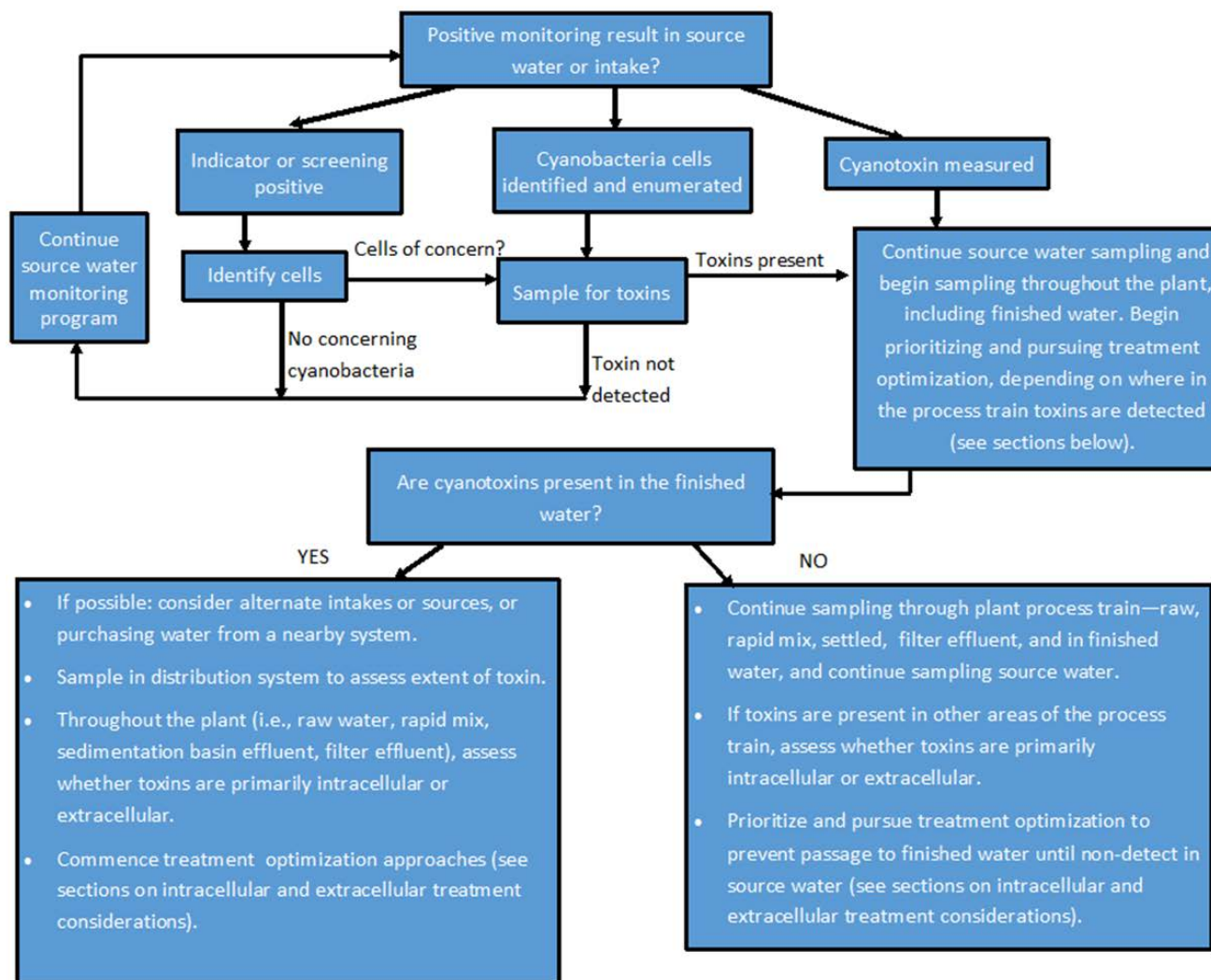
### Section 3: Treatment options based on source water quality

“Conventional” water treatment, defined here as having coagulation, clarification, and filtration processes, is typically effective at addressing intracellular cyanotoxins by removing the cyanobacteria cells (Health Canada, 2002). When a majority of cyanotoxins exist in the intracellular form (as is often the case with microcystins), and cells are not lysed, damaged, or stressed (Ross, et. al., 2006), conventional treatment processes are generally effective. However, if the cells become lysed or, in the case of cylindrospermopsin, which tends to partition between intracellular and extracellular closer to 50%-50% (AWWA, 2010), the appropriate approach may involve conventional treatment (i.e., coagulation, clarification and filtration) followed by an adsorption or oxidation step (NHMRC and NRMCC, 2011). Additionally, powdered activated carbon (PAC) may be added early in the treatment process to enhance extracellular toxin removal.

In order to evaluate treatment efficacy at a particular plant, regular monitoring and data trending, especially during HAB events, is important throughout the plant. Most of the parameters discussed above for source water monitoring may also be applicable indicators for the water treatment plant. However, once a HAB is confirmed and cyanotoxins are detected in the source water, daily cyanotoxin monitoring in the plant is the most prudent way of protecting public health and ensuring the quality of the finished water. For additional discussion of monitoring frequency, refer to Steps 4 and 5, and Figure 2 of “Recommendations for Public Water Systems to Prepare for and Respond to Cyanotoxins in Drinking Water” (U.S. EPA, 2015c).

Figure 3-1. Water treatment decision-tree for cyanotoxins detected in source water.

This figure begins with a “YES” answer to Step 3 of the “Recommendations” document decision-tree and is intended to provide more treatment detail to Steps 4 and 5 of that document.



Monitoring at multiple locations in the process train (the selection of which will depend on the plant configuration and chemical feed locations) can help water treatment plant operators evaluate the effectiveness of each unit process. This might include source water, raw water (with chemical addition if possible), recycle water feed, after chemical addition in the rapid mix (i.e., after chemicals are completely mixed), settled water, individual and combined filter effluent, and finished water. Once operators understand the performance of, and identify any limitations in each unit process, treatment adjustments can be made to improve toxin removal. The approach to making treatment adjustments for cyanotoxins depends on the monitoring results and type of cyanotoxins present (e.g., microcystins, cylindrospermopsin, or others). If cyanotoxins are present, it is helpful to understand if they are located within the cyanobacteria cell (intracellular) or outside the cell within the water matrix (extracellular). This can be accomplished through the analytical techniques mentioned above. Figure 3-1 depicts a suggested decision-tree<sup>10</sup> for water treatment plant operators that are currently monitoring their source water for cyanobacteria, cyanotoxins, and/or indicators.

### 3.1 Treatment considerations for intracellular cyanotoxins

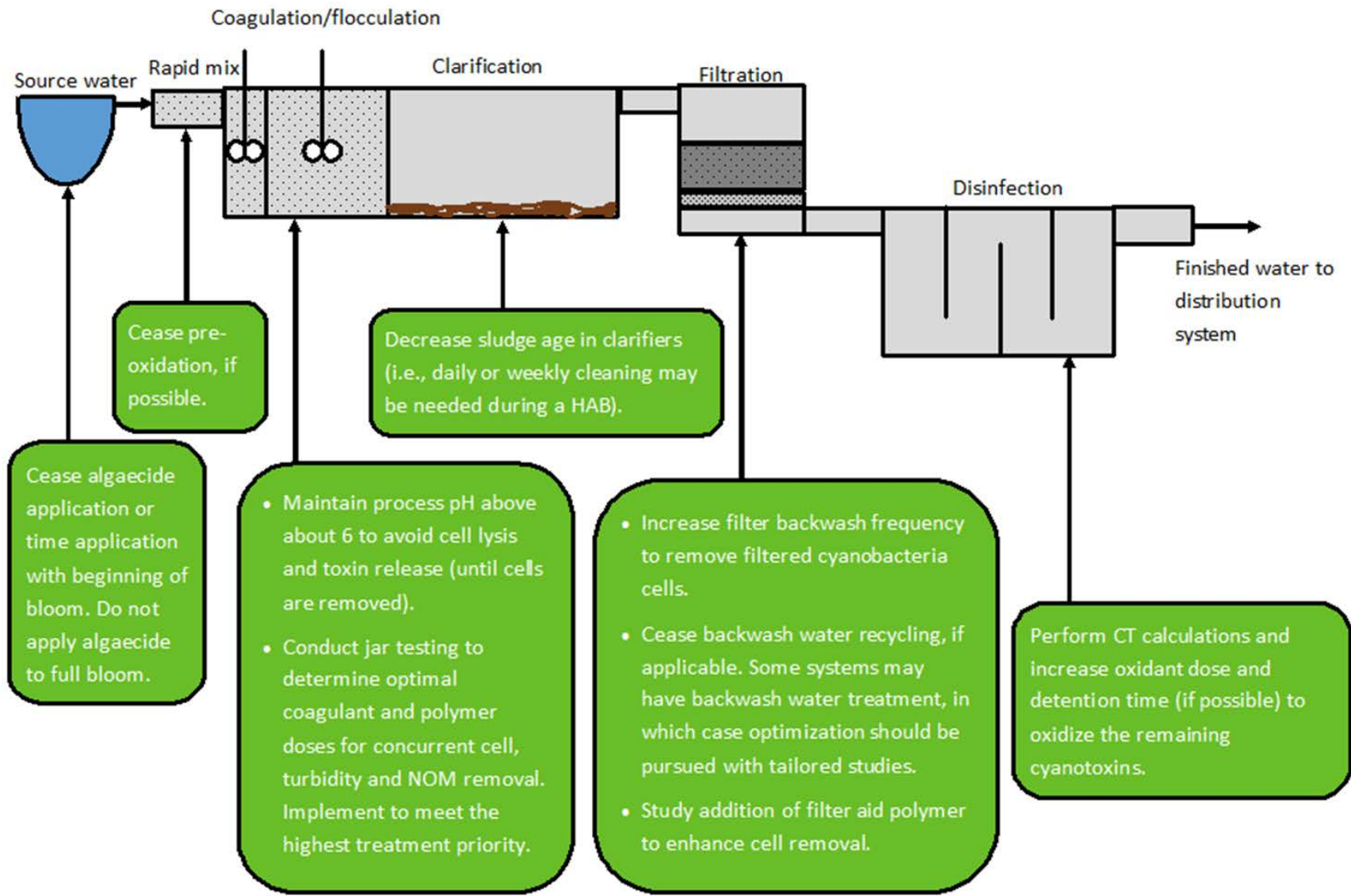
If cyanobacteria species and source water conditions are such that there is a significant fraction of toxins in intracellular form, a strategy that limits toxin release and maximizes cyanobacteria cell removal through the water plant should be considered. Specifically, operators would generally want to investigate their ability to stop or limit any pre-oxidation (i.e., oxidation prior to cell removal) and limit algaecide application, while focusing on optimizing their coagulation, clarification, and filtration processes for cyanobacteria cell removal. This may compromise other treatment objectives (i.e., manganese removal), that need to be considered while treating for cyanobacteria cell removal. Figure 3-2 depicts a plant schematic with treatment considerations for intracellular cyanotoxins at each process, which are discussed in more detail below

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<sup>10</sup> The decision-tree presented in “Recommendations for Public Water Systems to Prepare for and Respond to Cyanotoxins in Drinking Water” provides a framework for assessing vulnerability to, preparation for, monitoring for, and communication to stakeholders for HABs. The decision tree presented in the current document (Figure 3-1) provides specific treatment optimization strategies to guide water treatment plants that are already performing monitoring for HABs and that detect cyanobacteria or toxins in their source water or plant. In that sense, this fits into the treatment portion of Steps 4 and 5 of the “Recommendations” document’s decision-tree. Please refer to that document for details about establishing vulnerability assessments, preparation, monitoring programs, and communication to stakeholders for HABs.

Figure 3-2. Intracellular cyanotoxin treatment considerations

The focus of this figure on intracellular toxin treatment is on the physical removal of cells.



Although not depicted in Figure 3-2, microfiltration and ultrafiltration may also effectively address intracellular toxins by cell removal, without significant cell lysis and associated release of toxins into the water. Operational considerations if using micro- or ultrafiltration membranes for cyanobacteria cell removal during a HAB include evaluating backwash and cleaning frequencies due to plugging of screens and reduced flux and permeability. Microfiltration and ultrafiltration are not effective means of removing extracellular toxins however, as the molecular weight cutoff for these types of filters is greater than the molecular weight of the toxins (AWWA, 2010). For further discussion on membranes, see the “Treatment considerations for extracellular toxins” section below.

Operational considerations and potential studies for intracellular cyanotoxin removal through use of conventional water treatment are discussed below, and a more detailed checklist is provided in Table A-1 in the Appendices.

Operational considerations and potential studies:

A key component of an optimization program is process control and related problem solving activities. Plant staff can use tailored studies, based on the scientific method, to conduct in-house investigations on issues impacting performance. Below is a summary of relevant operational considerations and potential studies for water plants dealing with a HAB event. Implementing these strategies and conducting studies prior to a bloom will help plant operators be in a better position to respond to a cyanobacteria event.

- If a cyanobacteria bloom is detected in source water, operators can measure toxin concentrations in the clarifier effluent and compare concentrations at this location to raw water to determine if toxins are released prior to this location (e.g., settled cells, or in other upstream processes). If they are, consider additional sampling upstream to pinpoint the location of toxin release and respond appropriately through treatment (as discussed in Section 3.2) and consider tailored studies focused on reducing the release of cyanotoxins.
- In anticipation of reducing or stopping pre-oxidant use to minimize toxin release, studies can help assess and mitigate the impact of doing so on other treatment objectives that the pre-oxidant may be used to achieve (e.g., turbidity, TOC, and manganese removal; algae control in the plant; mussel control in intake line). Planning for and considering how these objectives will be achieved prior to the bloom season is critical.
- Optimizing coagulant and polymer dosing can maximize cell removal through the treatment process. This can be effectively evaluated in most plants using jar testing. Based on the literature (Newcombe, et al., 2015; Chow, et al., 1999; Henderson, et al., 2008; Vlaski, et al., 1996), NOM<sup>11</sup>, particle count, streaming current, zeta potential (Walker, 2015), UV, color, and/or pigment (i.e., phycocyanin or chlorophyll-*a*) may be useful parameters to evaluate during jar testing for optimization of the coagulation/sedimentation process. Also, coagulation pH will be an important variable to optimize. Given the variability that may occur with different coagulants and cyanobacteria species, operators should aim to maintain pH in a range between 6 and 8 through the plant for best cell removal and subsequent toxin oxidation.

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<sup>11</sup> Optimized NOM removal (defined as lowest  $\Delta C/C_0$  for DOC and UV, and color  $\leq 0.05$ ) resulted in optimized cyanobacteria cell removal in Newcombe, et. al. 2015.

- Operators should consider trending water quality data, including individual filter effluent (IFE), backwash water, and filter-to-waste (where applicable) turbidity values to understand baseline performance and compare during water quality challenges such as HABs. Trending settled water turbidity and cyanotoxin concentration during HABs will help to understand how the sedimentation process is performing.
- It is important to minimize the sludge age in clarifiers and increase the frequency of filter backwashing because settled and/or filtered cells can remain viable and possibly multiply over a period of at least 2-3 weeks. Within one day, cells in the sludge can lyse and release NOM and T&O compounds, in addition to cyanotoxins (Newcombe, et al., 2015). A water treatment plant should investigate options for design or operational modifications to enable more frequent cleaning.
- Operators who ensure that filters are optimized for turbidity removal and strive to achieve the optimization goal presented in the CCP Handbook (U.S. EPA, 2004) of  $\leq 0.10$  NTU in 95% of samples will generally be better prepared to deal with cyanotoxin challenges. Filter aid polymers may help cyanobacteria cell removal. Backwashing filters based on water quality data, such as effluent turbidity, rather than length of time in service can lead to more optimal filter operation. Studies using turbidity data can help operators optimize the backwash and filter-to-waste times, if applicable. High-rate backwash times may need to be extended in the event of a cyanobacteria bloom, while monitoring the filter so that it does not lose media. Utilities can also experiment with air scour, surface wash, and/or collapse-bed pulsing if applicable to their plant.
- Backwash water recycling during a HAB can be problematic. Studies can help operators understand the impacts of stopping backwash water recycling on raw water quality and backwash water disposal. Some systems may have backwash water treatment, in which case optimization can be pursued with tailored studies.

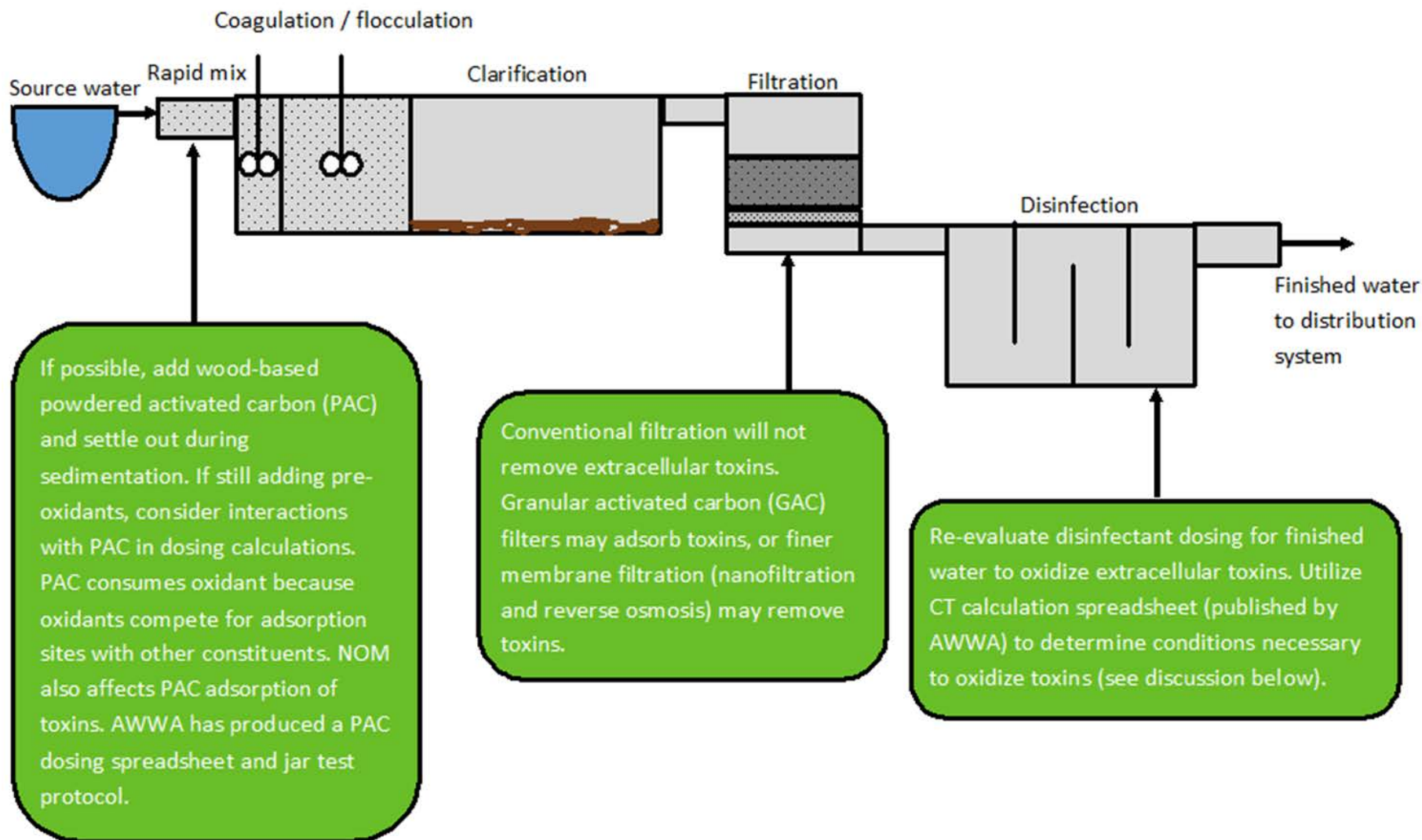
### 3.2 Treatment considerations for extracellular cyanotoxins

Because water utilities are designed to remove particulates as a matter of course, the preferred approach for water plants is to remove toxins while they are still in the intracellular form (i.e., within the intact cells), (AWWA, 2010). However, HABs can lyse in the environment, releasing the toxins into the extracellular, or dissolved, state. Also, some cyanobacteria species partition toxins between intracellular and extracellular states as a matter of course (as mentioned previously, for example, cylindrospermopsin tends to partition closer to 50%-50% extracellular-intracellular (AWWA, 2010)). From a treatment perspective, the use of a pre-oxidant will increase the risk of cell lysis or stress, which could lead to toxin release. Therefore, given the potential for extracellular toxins, specific treatment considerations to address them at each process are presented in Figure 3-3 and are discussed in more detail below the figure.



Figure 3-3. Extracellular cyanotoxin treatment considerations

The focus of this figure on extracellular cyanotoxin treatment is on adsorption, nanofiltration or RO, and oxidation.



### 3.2.1 Carbon adsorption

Carbon is often used at water treatment plants to remove taste and odor (T&O) and other organic compounds, typically in the form of powdered activated carbon (PAC) and granular activated carbon (GAC). Research has demonstrated effective removal of microcystins using PAC or GAC, and more limited research on the removal of cylindrospermopsin, anatoxin-a, and saxitoxin has also exhibited promising results (Walker, 2015). The type of carbon and corresponding mesopore size are important factors in determining the efficacy that carbon adsorption will have on the extracellular cyanotoxins. Wood-based or lignite-type carbon, with mesopores between 2-50 nanometers (nm) has been found to be most effective for microcystins (Walker, 2015; Ohio AWWA/Ohio EPA, 2015). However, this may challenge a competing objective of using carbon in the water treatment process for T&O compound removal, for which other types of carbon have been found to be most effective. Utilities should consider this if they need to utilize carbon for removal of multiple constituents. For example, utilities may consider using a mixture of carbons to remove cyanotoxins and T&O compounds.

T&O compounds, such as geosmin and methylisoborneol (MIB), NOM, and pre-oxidants such as permanganate and chlorine can affect activated carbon's ability to remove extracellular cyanotoxins. These compounds tend to compete for adsorption sites with the cyanotoxin molecules. Therefore, jar testing HAB-impacted raw water is an important step in optimizing not only coagulant/polymer, but also PAC dosing. Similarly, performing rapid small-scale column tests (RSSCTs) or accelerated column tests (ACTs) is an important step for understanding the empty-bed contact time (EBCT) and resulting media life, or adsorption capacity, for GAC.

#### 3.2.1.1 Powdered activated carbon (PAC)

PAC can be a short-term solution to cyanotoxin problems until more robust treatment can be installed. Short-duration PAC application can also be an effective long-term strategy for systems faced with seasonal cyanotoxin problems (repeated seasonal application) but lack the ability to install permanent treatment solutions (such as GAC or ozone). Potential PAC feed points can include the raw water intake, rapid mix prior to coagulation, or in clarifiers, depending on the application of other treatment chemicals (i.e., pre-oxidants), desired contact time and subsequent settling time. In a similar fashion to the CyanoTOX oxidant CT calculator, AWWA has also produced a "PAC Calculator for Cyanotoxin Removal", to be used in conjunction with the AWWA Cyanotoxin PAC Jar Testing Protocols<sup>12</sup>, to assist utilities in estimating an appropriate PAC dose during a HAB episode. However, because numerous factors impact performance, the optimal dose is best determined by jar testing with the actual water that contains the dissolved cyanotoxins. Generally, the most effective removals are achieved using wood-based PAC at contact times greater than about 45 minutes at a dose greater than 10 mg/L, although this is highly source water dependent (Alvarez, et al., 2010). Operational considerations and studies for PAC specific to cyanotoxin removal are discussed below, and a more detailed checklist is provided in Table B-1 in the Appendices.

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<sup>12</sup> AWWA's PAC Calculator for Cyanotoxin Removal and Cyanotoxin Jar Testing Protocols mentioned here, and the Cyanotoxin Tool for Oxidation Kinetics (CyanoTOX) mentioned in Section 3.2.4, can be found at AWWA's Cyanotoxins Resource Community website (login required).

As with any tool or model, it is important that the user understands the limitations and assumptions of that tool. AWWA has introductory tabs in each spreadsheet that discuss caveats and disclaimers for each tool. It is recommended that these pages be consulted prior to using each tool.

Operational considerations and studies for PAC specific to cyanotoxin removal:

- If PAC is only used seasonally, planning prior to bloom season should include ensuring sufficient supply of PAC and the ability to deliver and adequately mix a high PAC dose (i.e., greater than 10 mg/L). Utilities should also consider sufficient storage space and safety precautions as dust has explosive hazard properties.
- Utility planning for operational impacts of utilizing PAC on their sedimentation and filtration processes (i.e., more frequent sludge removal and disposal/treatment, potential for carbon fines on the filters, etc.) is important. PAC cannot be regenerated or recycled.
- Utilities considering using PAC should ensure that their plant's clarifiers are designed to allow PAC feed. PAC can cause damage to sludge rakes and mixers if they are not designed for settling and removing PAC.
- For plants that are not designed for feeding PAC, doses higher than about 10 mg/L may present performance challenges. High PAC doses can result in carbon fines passing through filters and increased filter effluent turbidity. Studies can be implemented to determine the optimum PAC dose that can be achieved while maintaining turbidity removal goals. The use of flocculant and filter aid polymers may be effective in enhancing PAC removal through sedimentation and filtration.

#### *3.2.1.2 Granular activated carbon (GAC)*

Utilities repeatedly affected by cyanotoxins in their source water may consider adding GAC filters. GAC has generally been shown to be effective for some cyanotoxin removal, especially using wood-based carbon (AWWA, 2010; Walker, 2015). Rapid small-scale column tests (RSSCTs) or accelerated column tests (ACTs) are valuable tools to determine if/when GAC is appropriate, to evaluate different carbon media types, and to ensure a best-fit for the particular utility and its source water. RSSCTs or ACTs also provide information on the necessary empty-bed contact time (EBCT) and the resulting media life before regeneration or replacement is needed. Media life will vary depending on numerous factors including carbon type, source water quality, and the desired effluent water quality. Due to the vagaries of bloom dynamics, it is difficult to conduct a RSSCT test that will be applicable to multiple blooms. Often the influent characteristics will differ from bloom to bloom in terms of concentrations and duration for both the toxins and other water quality parameters that can affect adsorption such as TOC concentration, temperature, pH, etc.

GAC is typically applied in one of two approaches. The first is as a filter adsorber where a portion of the sand bed (the top portion) is replaced with GAC. If an adequate flow distributor is chosen, all of the sand could be replaced with GAC. This allows the use of the existing sand filters. However, the depth of the bed is often limited to short EBCTs that can fail to remove all the cyanotoxins in the mass transfer zone resulting in early breakthrough. Particulate loading issues can also impact flow distribution, which would further exacerbate the limited EBCT. The other approach to applying GAC is to utilize a deep bed, where GAC column follows the sand filters, receiving the filter effluent. This is advantageous in that the GAC column depth can be designed independent of the existing sand filters and the column will not require as frequent backwashing as the sand filters. An increased EBCT will be more able to treat the cyanotoxin event. The disadvantage to installing separate GAC columns after the sand filters is that this is more capital intensive, as it requires the columns, plumbing, piping and instrumentation changes.

If GAC is pursued as an option, final design should consider that GAC filters are often used for more than one purpose, such as control of pesticides, herbicides, disinfection byproduct precursors, taste and odor events, and cyanotoxins. EBCTs reported in the literature typically range from 5 to 15 minutes, although some carbon vendors recommend at least a 10 minute EBCT. Adequate EBCT allows for greater removal of competing constituents such as TOC along with cyanotoxins, and allows for greater operational flexibility. Media life reported in the literature for cyanotoxin removal ranges from weeks to 6 months (Alvarez, et al., 2010), although this is highly site-specific. Operational considerations and studies for GAC specific to cyanotoxin removal are discussed below, and a more detailed checklist is provided in Table B-2 in the Appendices.

Operational considerations and studies for GAC specific to cyanotoxin removal:

- Consider media regeneration or replacement in routine maintenance schedules in preparation for the summer season.
- Evaluate operational considerations associated with GAC use. For example, not adding chlorine prior to the filters when they are in operation, as this affects adsorption capacity. Also, chlorine will be reduced in the top portion (about one inch) of the GAC bed, and hence need to be reapplied post-GAC filtration.
- If the GAC column is a filter adsorber, consider developing an adequate backwash procedure and schedule so as to:
  - Minimize washing GAC out of the filter during backwash,
  - Allow a uniform GAC layer to set up after backwashing, and
  - Minimize caking of the GAC particles with particulates and any biological growth present.
- Potentially, regeneration frequency could be based on the results of RSSCTs or ACTs conducted using the GAC media utilized by the plant and cyanotoxin of concern.
- Filter maintenance and flow distribution are important. Ensuring even media depth throughout the filter will mitigate preferential flow and potential breakthrough.

### 3.2.2 Membranes

Although rare in the drinking water industry in fresh water applications, the “tighter”, high-pressure membranes, reverse osmosis (RO) and nanofiltration, are capable of removing extracellular cyanotoxins by a combination of size exclusion and charge effects, depending on the cyanotoxin molecule being removed. (AWWA, 2010). Operational considerations and studies for high-pressure membranes specific to cyanotoxin removal are discussed below, and a more detailed checklist is provided in Table B-3 in the Appendices.

Operational considerations for membranes specific to cyanotoxin removal:

- Because RO and nanofiltration can remove extracellular cyanotoxins, the concentrate stream of these processes can have a high toxin retention level. Consider residual disposal issues that may arise due to high cyanotoxin concentrations (AWWA, 2010).

### 3.2.3 Biofiltration

Studies have demonstrated that biodegradation of a variety of cyanotoxins, including microcystins, nodularin, cylindrospermopsin, and anatoxin-a can occur in some situations. This is dependent on water temperature, the abundance of specific bacteria capable of degrading the cyanotoxins present, the

concentration of the target cyanotoxins, the presence of organic matter in the source water, and the presence of metals in the source water. Studies have also shown that the biodegradation products of saxitoxin may actually result in more toxic forms (Ho et al., 2012).

The most effective way for water treatment plants to utilize biodegradation for cyanotoxins is likely by biological filtration processes, or biofiltration. Water treatment plants can consider if it is feasible to modify existing sand or GAC filters to make them biologically active and able to host microorganisms that are capable of degrading the cyanotoxins that are present in their source water (Ho et al., 2012).

Operational considerations for biofiltration specific to cyanotoxin removal:

- Many studies have documented a lag period prior to the onset of biodegradation of cyanotoxins. This could be due to several reasons including time required for the population of organisms capable of degradation of the cyanotoxins present to reach sufficient numbers, or due to the time required for those organisms to induce the enzymes responsible for degradation of the cyanotoxins. This lag time is a significant consideration for utilities faced with cyanobacteria blooms that vary on short time periods.
- Both sand and GAC filters can be adapted to be biologically active, however some research suggests that GAC may be preferred, as two removal mechanisms would be applicable (adsorption and biodegradation). Refer to Section 3.2.1.2 on GAC for a discussion on the adsorption component of cyanotoxin removal. GAC may also be a better substrate for bacterial attachment than sand. Other types of media have shown promise for biofiltration in the research, including glass beads, porous ceramic, and plastic media. Media characteristics such as particle size, chemical composition, and roughness can influence the ability of the media to establish biological growth and biodegradation of cyanotoxins (Ho et al., 2012).
- If saxitoxins are the predominant cyanotoxin present in the source water, biofiltration may not be advisable, as research has demonstrated in some cases that the biodegradation products can be more toxic than the original cyanotoxin.
- Other factors that influence the effectiveness of biofiltration are filter contact time and hydraulic loading rate. Longer contact time and slower hydraulic loading rates may increase biodegradation of cyanotoxins.
- Utilities considering biofiltration should consider that prechlorination should not be performed prior to biological filtration processes. Carrying a chlorine residual onto the filters would impact the bacterial population and decrease cyanotoxin biodegradation. This may affect this option's feasibility in certain water treatment plants.
- Certain organisms are capable of biodegradation of certain cyanotoxins. Even if a filter has an active biofilm does not necessarily suggest that biodegradation of the specific cyanotoxins present will occur. Some preliminary research suggests that seeding filters with organisms capable of biodegrading the specific cyanotoxins present in a given source water may help to minimize the lag period and potentially increase the biodegradation. However, thorough analysis should be conducted prior to implementing this strategy to consider all potential impacts on other treatment objectives and resulting impacts on water quality.

#### 3.2.4 Oxidation

A variety of techniques exist for oxidation of cyanotoxins. The more common techniques, along with their particular advantages and limitations, are described in Table 3-1 and the subsections below.

Table 3-1. General effectiveness of cyanotoxin oxidation with common water treatment oxidants<sup>13</sup>

Oxidant	Anatoxin-a	Cylindrospermopsin	Microcystins	Saxitoxin
<b>Chlorine</b>	Not effective	Effective (at low pH)	Effective*	Somewhat effective
<b>Chloramine</b>	Not effective	Not effective	Not effective at normal doses	Inadequate information
<b>Chlorine dioxide</b>	Not effective at normal doses	Not effective	Not effective at normal doses	Inadequate information
<b>Potassium permanganate</b>	Effective	Data ranges from not effective to possibly effective	Effective*	Not effective
<b>Ozone</b>	Effective	Effective	Very effective	Not effective
<b>UV / advanced oxidation</b>	Effective	Effective	Effective at high UV doses*	Inadequate information

\* Dependent on initial cyanotoxin concentration, pH, temperature, and presence of NOM.

#### 3.2.4.1 Free chlorine

Research has demonstrated effective oxidation of microcystins and cylindrospermopsin by free chlorine (AWWA, 2010; Walker, 2015). Free chlorine is generally ineffective against anatoxin-a (AWWA, 2010).

The effectiveness of free chlorine oxidation of microcystins and cylindrospermopsin is pH dependent. As an example, Tables 3-2 and 3-3 show CT (chlorine concentration x contact time) values for microcystin-LR and cylindrospermopsin, respectively, to achieve the EPA Health Advisory lower levels (levels for infants to school-age children exposure, which are 0.3 µg/L and 0.7 µg/L, respectively) under varied pH, temperature, and cyanotoxin concentrations. These tables were developed using AWWA’s Cyanotoxin Tool for Oxidation Kinetics (CyanoTOX). AWWA’s stated purpose of the CyanoTOX tool is to “...provide water utilities with a means to assess how changes in their existing treatment (e.g., pH, oxidant dose, and contact time) will influence the degradation of specific cyanotoxins or groups of cyanotoxins.” As with any tool or model, it is important to understand the limitations and assumptions of that tool prior to its use (see footnote 12 at the bottom of page 20 for information on how to access this tool, which also provides information about the tool’s limitations). Utilizing this spreadsheet tool, parameters such as pH, temperature, cyanotoxin concentration, oxidant type (free chlorine, monochloramine, chlorine dioxide, and ozone are available), a plant-specific baffling factor, and cyanotoxin type can be varied to achieve a desired cyanotoxin target level. For this example, the results of which are shown in Tables 3-2 and 3-3, microcystin-LR and cylindrospermopsin targets were selected to correspond with the EPA Health Advisories issued for these toxins, and initial toxin concentrations were varied at certain intervals to provide the reader an idea of how the CT varies for free chlorine at various pH levels and temperatures. Please note that this is only an example, and that effects for different source waters, especially those containing compounds with competing oxidant demands, and different treatment may result in different CT values than those presented in Tables 3-2 and 3-3.

Although effects for different waters may vary, the results from this example (Tables 3-2 and 3-3) demonstrate that, particularly for microcystin-LR, the desirable pH range for the most efficient oxidation

<sup>13</sup> Adapted with permission from Ohio AWWA and Ohio EPA’s “White Paper on Algal Toxin Treatment” (Ohio AWWA & Ohio EPA, 2015).

with free chlorine is between 6 and 8. Some studies have shown that there is a risk of cell stress or lysis and toxin release at pH below 6 (Newcombe, et al., 2015). Above a pH of 8, particularly for microcystins, the resulting CT values may be unreasonably high, potentially resulting in plants exceeding the maximum residual disinfectant level (MRDL) for free chlorine or necessitating potentially unreasonably long contact times. Because this is a generic modeling example and there is no consideration for unique water qualities or the exact suite of cyanotoxin congeners, a utility should perform a similar evaluation for their own water quality with full understanding of the tool's assumptions and limitations.

Table 3-2. Microcystin-LR CT table

(For free chlorine to achieve the lower level [school-age child exposure] of the EPA Health Advisory)

**Toxin** = Microcystin-LR

**Oxidant** = Free chlorine

**Target** = 0.3 µg/L

pH	MC-LR conc [µg/L]	Effective CT [mg/L * min]				
		10°C	15°C	20°C	25°C	30°C
6	10	48.8	42.2	36.6	32.0	28.1
	25	61.5	53.2	46.2	40.4	35.4
	50	71.2	61.5	53.5	46.7	41.0
	100	80.8	69.9	60.7	53.0	46.5
7	10	56.9	50.1	44.3	39.5	35.5
	25	71.8	63.1	55.9	49.8	44.7
	50	83.1	73.0	64.7	57.6	51.7
	100	94.3	82.9	73.4	65.5	58.8
8	10	129.8	119.7	111.2	103.9	97.6
	25	163.7	151.0	140.2	131.0	123.1
	50	189.3	174.7	162.2	151.6	142.4
	100	215.0	198.3	184.2	172.1	161.7
9	10	466.6	421.7	382.0	346.8	315.3
	25	588.5	531.9	481.9	437.4	397.7
	50	680.7	615.3	557.4	505.9	460.0
	100	772.9	698.7	632.9	574.5	522.3

Table 3-3. Cylindrospermopsin CT table

(For free chlorine to achieve the lower level [school-age child exposure] of the EPA Health Advisory)

**Toxin** = Cylindrospermopsin

**Oxidant** = Free chlorine

**Target** = 0.7 µg/L

pH	CYL conc [µg/L]	Effective CT [mg/L * min]				
		10°C	15°C	20°C	25°C	30°C
6	10	10.3	8.1	6.5	5.2	4.2
	25	13.9	10.9	8.7	7.0	5.6
	50	16.6	13.0	10.4	8.3	6.7
	100	19.3	15.2	12.0	9.7	7.8
7	10	4.1	3.3	2.6	2.2	1.8
	25	5.5	4.4	3.6	2.9	2.4
	50	6.5	5.2	4.2	3.5	2.9
	100	7.6	6.1	4.9	4.0	3.3
8	10	8.1	6.9	5.9	5.2	4.5
	25	10.8	9.2	8.0	6.9	6.1
	50	12.9	11.0	9.5	8.3	7.3
	100	15.0	12.8	11.0	9.6	8.5
9	10	55.2	48.5	43.1	38.6	34.8
	25	74.2	65.3	57.9	51.9	46.8
	50	88.6	77.9	69.2	61.9	55.9
	100	102.9	90.6	80.4	72.0	64.9

#### 3.2.4.2 Ozone and advanced oxidation

Research has also demonstrated effective oxidation of microcystins and cylindrospermopsin by ozone (AWWA, 2010; Walker, 2015). However, specific CT values have yet to be reported (AWWA, 2010) and many factors play a role in ozone’s effectiveness, including pH, temperature, and concentration of NOM. In addition, the formation of oxidation byproducts with the use of ozone, such as bromate, should be considered. Advanced oxidation, such as ozonation at high pH, ozonation combined with hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), ferrous iron combined with hydrogen peroxide, or ultraviolet (UV) irradiation combined with hydrogen peroxide, has proved to be effective at treating extracellular microcystins, cylindrospermopsin, and anatoxin-a (AWWA, 2010).

#### 3.2.4.3 Chlorine dioxide and chloramines

Chlorine dioxide and chloramines have not been found to be as effective as alternatives at oxidizing certain cyanotoxins, including microcystins (AWWA, 2010; Walker, 2015).

For systems that use chloramines with minimal or no free chlorine contact time, advance HAB-response planning is warranted because of the limited effectiveness of chloramines. For example, AWWA’s M57 Manual, “Algae: Source to Treatment” cites a study that dosed 30 mg/L of monochloramine with a contact time of 5 days and was unable to degrade microcystins, with similar results for cylindrospermopsin.



#### 3.2.4.4 Potassium permanganate

Research indicates that potassium permanganate (KMnO<sub>4</sub>) is relatively effective against microcystin-LR and anatoxin-a (pH independent for microcystins, pH dependent for anatoxin-a), while ineffective against cylindrospermopsin. Studies using doses of approximately 1 mg/L have resulted in significant microcystins reduction (AWWA, 2010; Walker, 2015). However when used as a pre-oxidant, consideration should be given to the possibility of cyanotoxin release from cells. For example, some studies have shown that potassium permanganate causes release of toxins from cyanobacteria cells, especially at doses greater than 3 mg/L (Ou, et al., 2012), while other studies have shown limited to zero toxin release at doses below 3 mg/L (Fan et al., 2013a, 2013b, 2014). The potential for permanganates to cause release of cyanotoxins and other intracellular material may be at least partially dependent on the type of cyanobacteria present, possibly because some cyanobacteria have more robust cell walls that are more difficult to lyse.

Operational considerations and studies for oxidation technologies specific to cyanotoxin removal:

- Operational considerations and studies for oxidation technologies specific to cyanotoxin removal are discussed below, and a more detailed checklist is provided in Table B-4 in the Appendices.
- Consider what oxidants/disinfectants are available for use, their point of application, and any competing technologies that would limit their effectiveness.
- As with any type of oxidant, consider the potential for formation of regulated disinfection byproducts, total trihalomethanes (TTHMs) and haloacetic acids (HAA5s), bromate formation (from ozone), and the potential for cyanotoxin oxidation byproducts (Health Canada, 2015).
- Advance planning for utilities using monochloramine could include temporarily moving the point of ammonia addition further downstream in the treatment process train in order to allow more contact time with free chlorine to oxidize extracellular toxins during a HAB. Plants in this situation should consider evaluating the ability to move the point of ammonia addition and the effects that change may have on other water treatment objectives.

## Section 4: Implementing an optimization approach

This document, as well as other referenced resources, provides information that can be used strategically for addressing HABs. However, the challenges associated with implementing change in a treatment plant can be daunting. EPA's CCP has successfully utilized the following guidelines for implementing change in organizations since the inception of the program (U.S. EPA, 2004):

- Establish optimization goals that have the buy-in of utility staff and management.
- Create accountability by defining expectations of team members through clear roles and responsibilities, documentation of meeting outcomes, and assignment of action items.
- Use data-based decision-making to gain support from utility staff and management for making significant process changes (i.e., apply problem solving skills, such as tailored studies and data trending and interpretation).
- Develop operational policies and procedures to enhance communication among utility staff and management on critical activities (e.g., decision tree logic, when to sample for toxins, monitoring protocols).

- Establish routine communication (e.g., meetings, data distribution, memorandums) to continuously assess water system performance and provide a feedback loop.

Water utilities that apply this or a similar framework will likely have increased success in implementing the strategies presented in this document.

## Section 5: Conclusions

Increasing occurrence and detection of harmful algal bloom toxins in drinking water sources pose a variety of challenges for water treatment plant managers and operators. Optimizing water treatment processes for cyanotoxins, in conjunction with other water treatment objectives, can be daunting. However, monitoring procedures and process control tools that are already in place at many plants can be utilized for cyanotoxin treatment as well. A water treatment plant capable of optimization not only consists of good design, but also has supportive administration/management and is well-operated and maintained. A central theme to optimization for water treatment involves routine process monitoring and applying process control tools and problem solving skills based on that monitoring data. Several approaches to monitoring for cyanobacteria and resulting cyanotoxins were discussed. However, to evaluate the efficacy of each unit process in the water treatment process train, regular monitoring, especially during bloom seasons, is important throughout the plant. Water treatment plant operators should consider monitoring at multiple locations in the process train, such as in source water, after rapid mix, settled water, individual and combined filter effluent, or finished water. It is important to understand which type of cyanotoxin is present and whether the cyanotoxins reside within the cell or as extracellular because the optimal treatment approaches will differ. Plant optimization is never “finished” – it is an ongoing process; therefore, utilities are encouraged to enhance or modify their monitoring and treatment strategies as additional information becomes available through their regular source water and process monitoring, and through keeping up to date on research in the drinking water field.

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## Glossary

**ADDA.** 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid. An amino acid that is part of the microcystin molecule and is common to a majority of microcystin congeners.

**Congener.** Variants of similar molecules. For example, microcystin has five non-protein amino acids that are typically constant between variants, and the molecule has positions for two protein amino acids which can vary. These protein amino acids distinguish microcystin variants from each other. The different microcystin molecules are called congeners.

**CT.** Concentration x contact time. A measure of oxidation requirement for inactivating or oxidizing a contaminant.

**MMPB.** 3-methoxy-2-methyl-4-phenylbutyric acid. An oxidation product of microcystin that can be analyzed by LC/MS/MS. Shows promise as an analytical technique for total microcystin determination.

**Microcystin.** Sometimes abbreviated MC. Typically used in conjunction with its congener-specific amino acids (e.g. MC-LA, MC-LF, MC-LR, MC-LY, MC-RR, and MC-YR). The two letters after MC denote the specific amino acids (e.g. leucine and arginine = LR).

**MIB.** 2-Methylisoborneol. A common taste and odor chemical targeted by water treatment.

**PCR.** Polymerase chain reaction. A molecular DNA amplification technique that can be used to identify cyanobacteria and toxin-producing genes within the cell.

**UV<sub>254</sub>.** Water quality test to estimate organic material in drinking water samples; measurement is done utilizing UV light at 254 nanometers (nm).



## Appendix A: Process evaluation for treatment of intracellular toxins.

This appendix is intended for systems experiencing cyanobacteria blooms that have a significant portion of cyanotoxins in intracellular form. It can be used as a planning tool, or by systems in the midst of a bloom. The best strategy for controlling cyanotoxins will be system specific, but these tables can be used as a starting point to evaluate some common approaches. If the toxins are in both intracellular and extracellular form, these tables can be used in conjunction with the tables in Appendix B: *Process evaluation for treatment of extracellular toxins*.

It is important to ensure that proper process control monitoring plans are in place prior to implementing any treatment approaches for cyanotoxins, so that the impact and effectiveness of treatment can be assessed and informed treatment decisions can be made. Water treatment plant staff can design process control monitoring plans for cyanotoxins to best fit their situation (e.g., grab samples and/or online instruments depending on location, access, and availability of sampling ports). A good monitoring plan will include sampling for cyanotoxins if detected in the source water; surrogate parameters, as discussed in Section II of the main document; and other process control parameters specific to each technology (e.g., chemical dosing, feed rates, residuals, etc.).

It is also important to coordinate with the appropriate state or primacy agency prior to utilizing new or substantial changes in treatment in regard to that state's or primacy agency's permitting requirements.

Table A-1. Conventional treatment facility

Can my conventional treatment facility (coagulation, flocculation, and sedimentation) remove cyanobacteria cells / intracellular toxins?

Step	Question	If yes	If no	Comments/Notes
1.	<b>Source water:</b> Is an algaecide currently being added to the source water?	Evaluate ability to cease adding algaecide to minimize toxin release (e.g., via cell lysis or stress).	Continue to next step. Also, consider other source water control strategies summarized in the Comments/Notes column.	This assumes that the algaecide will result in release of the toxins (which research suggests is likely). Prior knowledge and frequent monitoring of intracellular and extracellular toxin levels will help determine the importance of ceasing algaecide application. Although control strategies in the source

Step	Question	If yes	If no	Comments/Notes
				<p>water will be system specific, there are other methods to reduce or mitigate the impact of cyanobacteria growth in the source water that may be applicable, such as:</p> <ul style="list-style-type: none"> <li>• Aeration to destratify the water column in the vicinity of the intake.</li> <li>• Switching to alternate intake level that may be less exposed to cyanotoxins.</li> <li>• Blending water with another source that is not exposed to cyanotoxins.</li> <li>• Raw water storage prior to a HAB, in anticipation of a future HAB.</li> <li>• If a bloom area is limited to a specific area or intake structure, consider bypass</li> </ul>

Step	Question	If yes	If no	Comments/Notes
				options (e.g., install temporary piping around the intake area or basin).
2	<b>Pre-oxidation:</b> Are pre-oxidants currently being added?	Evaluate ability to cease adding pre-oxidant or reduce the dosage due to the potential for cyanotoxin release. Ensure that other treatment objectives that are satisfied by pre-oxidant can be addressed.	Continue to next step.	Pre-oxidants are often used for a variety of water treatment objectives, such as turbidity, TOC, and manganese removal; algae control in the plant; or mussel control in intake lines. Advance planning allows one to consider how these objectives will be affected if pre-oxidation is stopped.
3	<b>Coagulation/flocculation/clarification:</b> Has the optimal coagulant dose been determined for the potential/current cyanobacteria bloom?	Continue to next step.	Review historical dosages that may have been effective in optimizing cyanotoxin control during past HABs, and conduct jar tests evaluating turbidity, NOM, UV254, pigments, and color removal as surrogates for cell removal (refer to the Operational considerations and	Optimized NOM removal (defined as lowest $\Delta C/C_0$ for DOC and UV, and color $\leq 0.05$ ) resulted in optimized cyanobacteria cell removal during jar testing (Newcombe et al., 2015). Jar testing may indicate that lower pH is needed for effective cell removal. Monitor pH

Step	Question	If yes	If no	Comments/Notes
			<p>potential studies section of Section III.A for more detailed discussion). Investigate the effectiveness of an alternate coagulant or flocculant aid polymer during the HAB event. (These studies should generally be performed through jar testing when possible).</p>	<p>and alkalinity to ensure optimized coagulation. See AWWA's M37: Operational Control of Coagulation and Filtration Processes for information on jar testing; monitoring; and coagulation, flocculation, and clarification process information<sup>1</sup>.</p>
4.	<p><b>Filter run time/backwash:</b> Have the filter run time and filter backwash disposal procedure been determined for the potential/current cyanobacteria bloom?</p>	Continue to next step.	<p>Consider impact of the HAB on filter run time (i.e., will it be shortened and what criteria will be used to initiate the backwash?). Assess the effectiveness of the filter backwash procedure for removing cyanobacteria cells (e.g., conduct studies on bed expansion, backwash waste water turbidity profile, and post-backwash filter recovery).</p>	

<sup>1</sup> American Water Works Association (AWWA). 2011. *Operational Control of Coagulation and Filtration Processes: Manual of Water Supply Practices M37*. 3rd edition. Denver, CO.

Step	Question	If yes	If no	Comments/Notes
			<p>Filter backwash disposal may be impacted (i.e., increased volume and contamination with cyanobacteria cells). How will this be addressed? Consider if the plant is recycling filter backwash water and if the recycle flow can be eliminated during the HAB event.</p>	
5	<p><b>Sludge management:</b> Does the plant have capacity to handle additional production and removal of settling basin sludge, or alter sludge handling practices (i.e., sludge recycling)?</p>	Continue to next step.	<p>Assess sedimentation basin sludge removal frequency and potential for toxin release from the sludge blanket. Determine the plant's capability for disposal of more frequent and increased amounts of sedimentation basin sludge. If applicable, assess operational and disposal implications of ceasing sludge recycling during a bloom.</p>	
6	<p><b>Filter Performance:</b> Are the filters optimized for turbidity removal (i.e. <math>\leq 0.10</math> NTU in 95% of turbidity samples)?</p>	Continue using conventional treatment to remove	Evaluate filter operational parameters with a goal to minimize effluent turbidity. For	

Step	Question	If yes	If no	Comments/Notes
		intracellular cyanotoxins.	example, see AWWA's M37: Operational Control of Coagulation and Filtration Processes <sup>2</sup> for information on monitoring and process control for filtration. Assess the condition of the filter media including media depth. Investigate the use of a filter aid polymer during the HAB event.	
7.				<b>Continue monitoring for cyanotoxins, reviewing and trending data, with diligence toward treatment to maximize cell removal and minimize toxin release in the plant. For extracellular cyanotoxin removal, see the tables in Appendix B.</b>

<sup>2</sup> American Water Works Association (AWWA). 2011. *Operational Control of Coagulation and Filtration Processes: Manual of Water Supply Practices M37*. 3rd edition. Denver, CO.

Table A-2. Membrane treatment process

Can my membrane treatment process (microfiltration [MF] or ultrafiltration [UF]) remove cyanobacteria cells / intracellular toxins?

Step	Question	If yes	If no	Comments/Notes
1.	<b>Performance:</b> Does direct or indirect integrity testing indicate any loss of particle removal effectiveness (LRV, pressure decay, turbidity, particle counts)?	<b>Short term:</b> Can the membranes that have lost integrity be cleaned/restored, replaced, or bypassed? If yes, go to the next question. <b>Long term:</b> Evaluate if replacing the affected modules is warranted.	Continue to next step.	
2.	<b>Concentrate disposal:</b> Is the reject water being sent to a location that can handle high cyanobacteria cell (and potentially toxin) concentrations? Note: if it is recycled and blended with fresh feed water, this will result in higher cyanobacteria cell (and possibly toxin) loading on the membranes.	Continue to next step.	Consider developing an alternative approach to handle the concentrate if there is concern for high cyanobacteria cell or cyanotoxin concentrations.	
3.	<b>Membrane backwashing:</b> can membranes be backwashed more frequently, if needed? Are backwashes initiated based on water quality, or some other membrane performance parameter?	Continue to next step.	Assess options for more frequent backwashing. Initiate backwash based on water quality or membrane performance parameter.	Backwash water will contain cyanobacteria cells. This may impact disposal practices.
4a.	<b>Membrane cleaning:</b> Can membranes be cleaned more frequently, if needed? Are clean-in-place (CIP) procedures that are initiated by water quality, or some	Continue to next step.	Initiate CIPs based on water quality or membrane performance parameters. Obtain a sufficient quantity of	

Step	Question	If yes	If no	Comments/Notes
	other parameter in place, and are the necessary chemicals available?		chemicals to clean the membranes if needed.	
4b.	<b>Cleaning solution disposal:</b> Are increased CIP and backwash cleanings anticipated as a result of the HAB?	Evaluate the capacity of the existing waste disposal system to handle the increase.	Continue to next step.	
5.	<b>Membrane pre-treatment:</b> Consider if there a need to adjust pre-treatment processes during a HAB?	Perform data-based studies to determine the necessary adjustments.	Continue with using MF or UF to remove intracellular toxins.	For example, pre-treatment that may need adjustment could include physical filters (pre-filtration – anticipate more frequent cleaning during HAB) and chemical feeds (if coagulants are added to remove organics for DBP control, the dose may need to be adjusted to also remove cyanobacteria – jar testing can help optimize the coagulant dose).
6.				<b>Continue monitoring for cyanotoxins throughout the process train, reviewing and trending data, and with diligence toward making treatment adjustments to reduce cyanotoxin concentrations in the finished water and process train.</b>



## Appendix B: Process evaluation for treatment of extracellular toxins.

These tables (arranged by treatment technology) are intended for systems with cyanobacteria blooms that have a significant portion of the cyanotoxins in extracellular form (i.e., outside the cell). The tables can be used as a planning tool, or by systems in the midst of a bloom. The best strategy for controlling cyanotoxins will be system specific, but these tables can be used as a starting point to evaluate some common approaches. Even if toxins are primarily intracellular, the tables in Appendix B can provide information on treatment for the fraction that exists as extracellular toxins; the tables can also be used to address situations involving toxin release due to algaecide or pre-oxidation. The treatment processes evaluated in Appendix B can be utilized in combination to increase the removal or destruction of cyanotoxins (particularly using post-oxidation as outlined in Table B-4). For removal of intracellular toxins, refer to Section 3.1 and Appendix A: *Process evaluation for treatment of intracellular toxins for treatment considerations for intracellular toxins*.

It is important to ensure that proper process control monitoring plans are in place prior to implementing any treatment approaches for cyanotoxins, so that the impact and effectiveness of treatment can be assessed and informed treatment decisions can be made. Water treatment plant staff can design process control monitoring plans for cyanotoxins to best fit their situation (e.g., grab samples and/or online instruments depending on location, access, and availability of sampling ports). The monitoring plan should include sampling for cyanotoxins if detected in the source water; surrogate parameters, as discussed in Section 2 of the main document; and other process control parameters specific to each technology (e.g., chemical dosing, feed rates, residuals, etc.).

It is also important to coordinate with the appropriate state or primacy agency prior to utilizing new or substantial changes in treatment in regard that state’s or primacy agency’s permitting requirements.

Table B-1. Powdered activated carbon (PAC)

Can my facility use PAC to treat extracellular cyanotoxins?

	Question	If yes	If no	Comments/Notes
1.	<b>PAC equipment:</b> Is PAC feed equipment currently in-place, or could it be installed in a short period of time (i.e., 24-48 hours)?	Continue to next step – for both immediate (short-term) and longer-term implementation of PAC.	Is this a long-term strategy that warrants pursuing (i.e., possibly for the next bloom season)?  If PAC feed equipment is not available in short order, other treatment strategies should be considered for removing extracellular	Document immediate and/or longer-term equipment needs, if applicable.  New PAC feed equipment should generally be piloted for short periods of time prior to implementing on a full-time basis in order to understand the plant’s response to the new

	Question	If yes	If no	Comments/Notes
			toxins (see Section 3.2 in the document and the other tables in Appendix B for alternative strategies).	equipment and applied dosage rates.
2.	<b>PAC dose:</b> Based on the anticipated influent cyanotoxin concentration, has the optimal PAC dose been determined to achieve treatment objectives?	Implement dosing protocol as determined and continue to next step.	Use AWWA’s PAC calculator and jar testing protocol, the activated carbon supplier’s recommendation, or primacy agency’s recommendation to estimate an initial optimal PAC dose. Follow up with process control monitoring and dose adjustments to optimize the removal of cyanotoxins (or surrogate parameters).	T&O compounds, NOM, and pre-oxidants such as permanganate and chlorine can affect activated carbon’s ability to remove extracellular cyanotoxins. Filter breakthrough of PAC fines may occur as the PAC dose is increased. This may limit a plant from feeding the recommended dose to achieve toxin removal targets. Consider approaches for mitigating this response if it occurs (e.g., adding a filter aid polymer). Is feeder capacity adequate for higher feed rates necessary for HABs? Coordinate with state prior to utilizing new or substantial changes in treatment in regard to state’s permitting requirements.
3.	<b>PAC type:</b> Has the optimal type of PAC for cyanotoxin adsorption been identified?	Use optimal PAC as previously determined and continue to next step.	Determine optimal type of PAC (i.e., as recommended by manufacturer, evaluated through jar testing, used by a neighboring system) and any potential supply issues/limitations.	See discussion of carbon types in Section 3.2.1. Wood-based carbon has been found to be effective for cyanotoxins but may not be as effective as other carbon types for T&O removal. A mixture of carbon types may

	Question	If yes	If no	Comments/Notes
				be necessary to achieve all treatment objectives during a HAB.
4.	<b>PAC supply:</b> Is an adequate supply of PAC on-hand? Can more be obtained quickly (i.e., 24-48 hours), if needed?	Take measures to ensure a continued adequate supply for the duration of the HAB and continue to next step.	Is this a long-term strategy that warrants pursuing (i.e., possibly for the next bloom season)? If yes, continue with this strategy. If PAC supply is not available in short order, other treatment strategies should be considered for removing extracellular toxins (see Section 3.2 in the document and the other tables in Appendix B for alternative strategies).	Document immediate and/or longer-term chemical supply needs, if applicable. Consider storage/space needs and safety in handling/storage.
5.	<b>PAC feed and locations:</b> Are there locations in the plant that are practical for feeding PAC at the feed rate necessary to achieve the needed dose?	Evaluate the next two questions for optimal location.	Consider a different approach to removing extracellular toxins (see Section 3.2 in the document and the other tables in Appendix B for alternative strategies).	Ensure that adequate mixing of PAC can be provided.
5a.	<b>PAC contact time:</b> Can PAC be added in the plant to allow enough contact time for significant adsorption? Has this been tested?	Use optimal location as determined and continue to next step.	It may not be effective to use PAC. Consider alternative approaches for extracellular toxin removal (see Section 3.2 in the document and the other tables in Appendix B for alternative strategies).	See AWWA's "PAC Calculator for Cyanotoxin Removal", to be used in conjunction with the AWWA Cyanotoxin PAC Jar Testing Protocols, to assist in estimating an appropriate PAC dose (CT) during a HAB episode.

	Question	If yes	If no	Comments/Notes
5b.	<b>Pre-oxidation:</b> Are pre-oxidants used that may affect PAC performance?	Stop pre-oxidants, if possible. Pre-oxidants such as permanganate and chlorine can affect activated carbon's ability to remove extracellular cyanotoxins, and activated carbon will reduce the oxidant concentration, possibly rendering it ineffective as well. Evaluate if pre-oxidation can be stopped and ensure that other treatment objectives satisfied by pre-oxidant can be addressed. If not possible, consider alternative approaches to removing extracellular toxins (see Section 3.2 in the document and the following tables in Appendix B for alternative strategies).	Continue to next step.	Pre-oxidants are often used for a variety of water treatment objectives, such as turbidity, TOC, and manganese removal; algae control in the plant; or mussel control in intake lines. Advance planning allows one to consider how these objectives will be affected if peroxidation is stopped. Consider whether it is practical to forego meeting some objectives (e.g., mussel control) for a short period of time while addressing the more immediate cyanotoxin issues.
6.	<b>Residuals:</b> Can the system handle additional sludge from settling basins and more frequent filter backwashes?	Continue with using PAC to remove extracellular cyanotoxins.	Estimate the quantity of sludge to be produced under the PAC treatment scheme based on the AWWA PAC calculator and evaluate the sludge disposal capacity of the plant. Can these issues be addressed,	

Question	If yes	If no	Comments/Notes
		or will this preclude the use of PAC?	
7.			<b>Continue monitoring for cyanotoxins throughout the process train, reviewing and trending data, with diligence toward making treatment adjustments to reduce cyanotoxin concentrations in the finished water and process train.</b>

Table B-2: Granular activated carbon (GAC)

Can my facility use GAC to treat extracellular cyanotoxins?

Question	If yes	If no	Comments/Notes
1. <b>Empty Bed Contact Time (EBCT):</b> Is there a GAC filter on line that has enough EBCT to control a cyanotoxin event (filter adsorber or post-filter adsorber)?	Continue to next step.	<b>Short term:</b> Because the implementation of a GAC technology takes a significant time to plan, permit, and construct; consider a different approach to removing extracellular toxins (see Section 3.2 in the document and the other tables in Appendix B for alternative strategies). <b>Long term:</b> Utilities repeatedly affected by cyanotoxins in their source water may wish to	Rapid small-scale column tests (RSSCTs) or accelerated column tests (ACTs) are tools for evaluating adsorption capacity and EBCT for long-term implementation studies. See Section 3.2.1.2 for discussion on GAC.

	Question	If yes	If no	Comments/Notes
			consider adding GAC filters as a long-term solution. Several factors such as plant design, financial, and source water quality play a role in this decision.	
2.	<b>Media life:</b> Is there adequate adsorption capacity remaining in the GAC?	Continue to next step.	Can the GAC be replaced quickly? If yes, go to the next question. If not, consider a different approach to removing extracellular toxins (see Section 3.2 in the document and the other tables in Appendix B for alternative strategies).	Media life will vary depending on numerous factors including carbon type, source water quality, length of prior operation, and the desired effluent water quality. RSSCTs and ACTs are effective tools for evaluating adsorption capacity.
3.	<b>Backwashing:</b> Are operational procedures in place to backwash the GAC filter(s) and dispose of the backwash water on a more frequent basis, if needed?	Continue with using GAC for removing extracellular cyanotoxins.	Develop backwashing policies, which include criteria for initiating a backwash, and planning for disposal of backwash water.	
4.				<b>Continue monitoring for cyanotoxins throughout the process train, reviewing and trending data, and with diligence toward making treatment adjustments to reduce cyanotoxin concentrations in the finished water and process train.</b>

Table B-3: High-pressure membranes (reverse osmosis [RO] and nanofiltration [NF])

Can my facility use high-pressure membranes (i.e., reverse osmosis [RO] or nanofiltration [NF]) to treat extracellular cyanotoxins?

	Question	If yes	If no	Comments/Notes
1	<p><b>Membrane Type:</b> Is there a membrane system on line that can remove cyanotoxins (RO or NF)?</p>	Continue to next step.	<p><b>Short-term:</b> Because the implementation of a high-pressure membrane system takes a significant time to plan, permit, and construct, consider a different approach to removing extracellular toxins (see Section 3.2 in the document and the other tables in Appendix B for alternative strategies).  <b>Long-term:</b> Assess if RO or NF are feasible options for cyanotoxin removal. RO/NF could be a feasible option if it helps meet multiple other treatment objectives.</p>	This table is mainly intended to provide treatment considerations for those systems that already have RO or NF in place and are affected by a HAB. Given that RO and NF tend to be expensive and complex/resource-intensive, systems will likely find that adding RO or NF membranes to their facility is cost/resource-prohibitive
2	<p><b>Performance:</b> Does the membrane exhibit chemical rejections (salts, TOC, specific chemicals) that are indicative of maintaining its original integrity?</p>	Continue to next step.	Can the membranes that have lost integrity be cleaned/restored, replaced, or bypassed? If yes, go to the next question. If not, consider a different approach to removing extracellular toxins (see Section 3.2 in the document and the	

	Question	If yes	If no	Comments/Notes
			other tables in Appendix B for alternative strategies).	
3.	<b>Concentrate disposal:</b> Is the reject water being sent to a location that can handle high cyanotoxin concentrations?	Continue to next step.	Develop an approach to handle concentrate. If not possible, consider a different approach to removing extracellular toxins (see Section 3.2 in the document and the other tables in Appendix B for alternative strategies).	
4a.	<b>Membrane cleaning:</b> Can membranes be cleaned more frequently, if needed? Are clean-in-place (CIP) procedures that are initiated by water quality, or some other parameter in place, and are the necessary chemicals available?	Continue to next step.	Initiate CIPs based on water quality or membrane performance parameters. Obtain a sufficient quantity of chemicals to clean the membranes if needed. If this cannot be done before the membrane fouls, consider a different approach to removing extracellular toxins (see Section 3.2 in the document and the other tables in Appendix B for alternative strategies).	
4b.	<b>Cleaning solution disposal:</b> Are increased CIP and backwash cleanings anticipated?	Evaluate the capacity of the existing waste disposal system to handle the increase.	Continue with using RO or NF for removing extracellular cyanotoxins.	
5.				<b>Continue monitoring for cyanotoxins throughout the</b>



Question	If yes	If no	Comments/Notes
			<b>process train, reviewing and trending data, and with diligence toward making treatment adjustments to reduce cyanotoxin concentrations in the finished water and process train.</b>

Table B-4: Oxidation: Can my facility use oxidation to treat extracellular cyanotoxins?

The oxidants free chlorine, chloramine, permanganate, ozone and chlorine dioxide are covered in AWWA’s CyanoTOX tool for determining CT. See Section III.B.3.0 for discussion on these and other oxidants, such as advanced oxidation processes (AOP). These tables focus on free chlorine and chloramine due to their prevalence in the water treatment industry.

Step	Question	If yes	If no	Comments / Notes
1.	<b>Optimal oxidant and application point:</b> Have studies been performed to determine the optimal oxidant and application location?	Continue to question 5.	Continue to question 2.	
2a.	<b>Primary disinfectant:</b> Does the plant currently use free chlorine as a primary disinfectant (i.e., to achieve CT)?	Continue and remain mindful of other treatment objectives (e.g., moving the point of chlorination to prior to removing TOC may increase DBP formation).	Continue to next step.	See Section 3.2.4 for discussion on other oxidants, such as ozone, permanganate, chlorine dioxide, and advanced oxidation processes (AOP).
2b.	<b>Secondary disinfectant:</b> Is chlorine used as the secondary disinfectant (i.e., for distribution system residual)?	Continue to next step.	For systems that use chloramine with minimal/no free chlorine contact time, advance planning is important to best respond to HABs because chloramines are	See Section 3.2.4 for discussion on other oxidants, such as ozone, permanganate, chlorine dioxide, and advanced oxidation processes (AOP).

Step	Question	If yes	If no	Comments / Notes
			generally not as effective at oxidizing cyanotoxins. See discussion in Section 3.2.4.3.	
3.	<b>Oxidant feed systems:</b> Is there access to equipment to feed oxidants within the next 24 hours?	Continue to next step.	<b>Short term:</b> If there is not enough time to obtain equipment, consider a different approach to treating extracellular toxins (see Section 3.2 in the document and the other tables in Appendix B for alternative strategies). <b>Long term:</b> Assess if additional oxidation is a feasible option for cyanotoxin removal. Investigate types of oxidants, equipment, and feed locations.	
4a.	<b>Practical feed location:</b> Are there locations in the plant that are practical for feeding oxidants?	Evaluate the next two questions for optimal location. Consider possible unintended consequences related to feeding oxidants (i.e., applying chlorine prior to TOC removal may increase DBP formation).	<b>Short term:</b> If feed points cannot be added in time, consider a different approach to treating extracellular toxins (see Section 3.2 in the document and the other tables in Appendix B for alternative strategies). <b>Long term:</b> Assess if additional oxidation is a feasible option for cyanotoxin removal.	

Step	Question	If yes	If no	Comments / Notes
			Investigate types of oxidants, equipment, and feed locations.	
4b.	<b>Contact time:</b> Have the locations that provide sufficient contact time been evaluated?	Use optimal location as previously determined	Identify and utilize a feed location that maximizes contact time and that doesn't compromise other treatment objectives (e.g., increase DBP formation).	AWWA's CyanoTOX tool can be used to estimate CT for cyanotoxins. See Section 3.2.4 for a more detailed discussion.
5.	<b>Oxidant dose:</b> Based on influent concentration, pH, and previous experience, has the optimal oxidant dose been determined?	Continue to next step.	Use AWWA's CyanoTOX tool (which can estimate CT for cyanotoxins) or follow state's recommendation to estimate optimal oxidant dose. Further process control monitoring of cyanotoxins (or surrogate parameters) can help to determine the optimal dose.	
6.	<b>Unintended consequences:</b> Are there any technologies currently used in the plant (e.g., PAC, GAC, membranes) that would be detrimentally impacted by enhancing the use of oxidation?	Consider adjusting the planned oxidation approach to avoid unintended consequences, or consider another approach to treating extracellular toxins if unable to adjust.	Continue to next step.	

Step	Question	If yes	If no	Comments / Notes
7.	<b>Oxidant supply:</b> Is there an adequate supply of the oxidant to deliver it at the intended dose?	Begin oxidant dosing, at optimal dose and location as previously determined	<b>Short term:</b> If time allows during the current HAB, purchase oxidant. <b>Long term:</b> Plan for future HABs by ensuring adequate supply of desired oxidants prior to bloom season.	
8.				<b>Continue monitoring for cyanotoxins throughout the process train, reviewing and trending data, and with diligence toward making treatment adjustments to reduce cyanotoxin concentrations in the finished water and process train.</b>