CYANOBACTERIA ARE PHOTOSYNTHETIC bacteria that are common in all freshwater and marine environments. They were historically called blue-green algae but their structure, genetics, and physiology clearly identify them as bacteria. Cyanobacteria in freshwater systems are widely recognized as sources of toxins (cyanotoxins) and unpleasant tastes and odors in water supplies. Cyanobacteria are a normal component of the natural biota and tolerate a wide range of climatic conditions and environments. A rise in the number of cyanobacterial blooms, caused by eutrophication from decaying plant materials and man-made pollution, is resulting in the production of more taste and odor compounds and natural toxins, which demands the attention of water treatment authorities. Although cyanotoxins are less commonly found in drinking water than taste and odor compounds, their high toxicity is of great concern. Due to global climate change, toxin-producing cyanobacteria are spreading into more temperate regions and becoming a more widespread problem.

Currently, there are no U.S. Environmental Protection Agency (EPA) regulations for cyanotoxins. However, three cyanotoxins are included on the final contaminant candidate list (CCL3): anatoxin-a, microcystin-LR, and cylindrospermopsin. At present, the only cyanobacterial toxin class that has been internationally assessed for health risk is the microcystins. The World Health Organization (WHO) has issued a provisional guideline value of 1 microgram per liter (µg/L) for microcystin-LR in drinking water and many countries have developed their own guidelines, depending on the types of cyanotoxins found in their source waters. The 2014 cyanotoxin event in Toledo has sparked significant regulatory activity. In June 2015, EPA issued 10-day Health Advisories (HAs) for states and utilities to protect the public from cyanotoxins in drinking water. For children younger than school age, the HA was set at 0.3 µg/L for total microcystin and 0.7 µg/L for cylindrospermopsin. The corresponding values for adults are 1.6 µg/L and 3.0 µg/L, respectively. Although not enforceable, the published HAs are intended to trigger utility actions including increased monitoring, development of treatment strategies, and public notification of “do not drink/do not boil” advisories. EPA has also provided recommendations on how utilities can monitor and treat drinking water for cyanotoxins. Additionally, the EPA has released a pre-publication copy of its proposed Fourth Unregulated Contaminant Monitoring Rule (UCMR4), which includes 10 cyanotoxins/groups.

Due to the increase of cyanobacterial blooms, the occurrence of several toxic metabolites (i.e., cyanotoxins) in water supplies has also increased. There is growing concern about the potential for negative health effects in humans and animals due to these toxins. These toxins enter water supplies through natural production and metabolic activities, and through cell lysis and subsequent release of toxins. Water collection and treatment activities may contribute to the release of cyanotoxins.

Presently, about 3,000 species of cyanobacteria are known; however, not all produce toxins. The organisms most frequently associated with toxin production are Microcystis, Oscillatoria, Cylindrospermopsis, Anabaena, Planktothrix, Aphanizomenon, Nodularia, and Lyngbya. Most poisoning by the cyanobacteria listed above involves three types of toxins (specific toxic compounds are listed in parentheses):

1. Hepatotoxins (microcystin [usually microcystin-LR and microcystin-LA], cylindrospermopsin, and nodularin), which are taken up by the liver and cause weakness and anorexia.
2. Neurotoxins (usually anatoxin and saxitoxin), which affect the nervous system.
3. Dermatoxins (aplysiatoxin and lyngbyatoxin), which cause skin and mucous irritations upon contact.

**Taste and Odor Compounds and Toxic Cyanobacteria**

THE MOST FREQUENTLY cited cyanobacterial metabolites are geosmin and 2-methylisoborneol (MIB). Geosmin and MIB impart unpleasant earthy/musty odors to the water. Attempts to use taste and odor parameters as potential indicators of the presence of toxins have been inconclusive. Just as it is true that most cyanobacterial species do not cause taste and odor problems, it is also true that most do not produce toxins. Some species that produce taste and odor compounds, however, can also produce toxins.

**The Water Research Foundation (WRF) and Cyanotoxin Research**

WRF HAS SPONSORED research on toxic algae since 1993, initially producing a comprehensive resource guide for utilities: **Cyanobacterial (Blue-Green Algal) Toxins: A Resource Guide** (project #925). Since the publication of the guide, WRF has funded additional research on control, treatment, and detection methods for cyanotoxins, much of it in collaboration with research partners.

The increased frequency of cyanobacterial blooms in the United States prompted WRF, in partnership with the EPA, to fund one of the first projects to investigate cyanotoxins as a potential threat to U.S. water systems. Published in 2001, the comprehensive study, **Assessment of Blue-Green Algal Toxins in Raw and Finished Drinking Water** (project #256), assessed microcystin occurrence and treatment removal capabilities. During the project, 45 utilities in the United States and Canada were surveyed for two years during cyanobacterial blooms. Microcystin was found in 80% of the source waters. Only two of the finished water samples were above the WHO guidelines (1 µg/L). The study also showed that almost all utilities had adequate procedures to reduce microcystin to safe levels in finished water.

In 2014, WRF and the American Water Works Association (AWWA) co-sponsored project #4548, **Cyanotoxin Guides for Water Utility Managers**, which distilled and summarized information from the last 20–25 years of cyanotoxin research. This...
purpose of this project was to develop two cyanotoxin utility action guides designed for use by water utility management and personnel, and the drinking water community. These guides were intended to synthesize existing knowledge on cyanobacterial and cyanotoxin events and provide practical tools to help the water community prepare for, and respond effectively to, potential events.

The first manual, *A Water Utility Manager’s Guide to Cyanotoxins* (project #4548a), is a succinct summary of cyanotoxin occurrence trends, potential health effects, preemptive and mitigation strategies, and potential challenges. This guide is intended to help water utility managers recognize if cyanotoxins may be an issue for their utility and what initial steps to consider. A short self-assessment allows utility managers to assess the vulnerability of their water systems to cyanobacteria and cyanotoxin events and, when needed, where they can find additional information, available resources, and necessary guidance.

The second guide, *Managing Cyanotoxins in Drinking Water: A Technical Guidance Manual for Drinking Water Professionals* (project #4548b), is an action-oriented synthesis of relevant literature for utility personnel and the water utility community, providing information necessary to guide the development of a technically sound evaluation of cyanotoxins as a water quality concern for drinking water supplies and appropriate mitigation measures. The guide summarizes the most recent information about cyanotoxin occurrence, analytical methods and monitoring, and management strategies. Like the first guide, it is also intended to benefit water utility managers, operators, and consultants. More specifically, though, it is intended for users working for, or with, water utilities that have already been determined to be at risk of having cyanobacteria and possibly cyanotoxin issues. While this second guide contains more detailed information than the first guide, it is organized to help readers navigate the issues and make informed decisions about appropriate mitigation measures and how to be prepared in case of toxic cyanobacteria blooms.

**Treatment**

THERE ARE SEVERAL conventional and advanced treatment options available for the removal of cyanotoxins. The key is in understanding the specific toxin of concern, because different toxins are removed/inactivated at varying degrees by different treatment technologies. For example, several research studies indicate that water treatment plants that meet Stages 1 and 2 of the Disinfectants/Disinfection By-product Rule by using ozone have a considerable level of protection from several types of cyanotoxins (such as microcystin), but not from saxitoxin. On the other hand, pre-oxidants such as potassium permanganate, ozone, and chlorine (which are used to mitigate cyanobacteria) have been found to lyse cyanobacteria, which releases toxins; therefore, it is recommended that coagulation be used prior to oxidation to remove whole cells.

One of the first WRF projects to address the removal of cyanotoxins through water treatment was conducted as a Tailored Collaboration project with United Water International (Australia). The report, *Removal of Algal Toxins from Drinking Water Using Ozone and GAC* (project #446), was published in 2002. The researchers conducted lab and pilot plant tests for the control of cyanotoxins through treatment (ozone, granular activated carbon [GAC], biological filtration) to assess the optimal conditions under which microcystin, anatoxin-a, and saxitoxin are inactivated. It was found that ozone is an efficient treatment for anatoxin-a and microcystin. However, saxitoxin is not readily destroyed under the same conditions. The study also determined that GAC adsorption is not effective for the removal of microcystins. However, a later study demonstrated that GAC is effective if it is replaced frequently. In addition, excellent removal is achieved when GAC is operated in the biological mode. Effective removal of toxicity was found with GAC for saxitoxin. Biological filtration was not effective for saxitoxin.

Building on this project as well as other research, a Tailored Collaboration project with the City of cocoa (Fla.), *Treatability of Algal Toxins Using Oxidation, Adsorption, and Membrane Technologies* (project #2839), was funded to identify and assess viable control and treatment methods, including design, operating criteria, and estimated treatment costs, to mitigate microcystin-LR in finished water. The study conducted bench-scale tests for the following treatment technologies: UV/H₂O₂, ozone/AOP, powdered activated carbon (PAC), GAC, biological degradation, and membranes (reverse osmosis [RO] and nanofiltration [NF]).

UV/H₂O₂ was effective but was dictated by H₂O₂ concentrations and availability; UV alone was not effective. The study found GAC was effective at removing microcystin when GAC was replaced frequently and total organic carbon concentrations were low. The ozone/AOP combination was effective at removal, mostly if pH was below 7; doses as low as 0.4 mg/L achieved microcystin-LR removal greater than 97%. PAC was also able to remove microcystin-LR at doses of 10 mg/L and with a contact time of 30 minutes. Biological degradation provided 35% removal of microcystin-LR; thus, it was recommended that it should be used as a polishing step in conjunction with other treatment methods, such as UV, oxidation, ozonation, PAC, and GAC. The RO and NF membranes tested removed microcystin-LR efficiently, at a minimum of 95%. Based on the effectiveness of the technologies above, costs of implementation and engineering considerations are provided in the report for utilities to make suitable treatment decisions.

Project #4016, *Evaluation of Integrated Membranes for Taste and Odor and Algal Toxin Control*, was a partnership with the Australian Cooperative Research Center for Water Quality and Treatment (CRCWQT). This project studied the feasibility of membrane technologies (ultrafiltration [UF], NF, and RO) in conjunction with coagulation, PAC, or microfiltration (MF) membranes for the removal of taste and odor compounds and cyanotoxins. The study focused on the removal of extracellular...
MIB, geosmin, cylindrospermopsin, and the major microcystin variants by RO and NF. The project evaluated a UF integrated membrane system (IMS) ideally suited to the removal of dissolved algal metabolites (microcystins, geosmin, MIB, and cylindrospermopsin). The removal of cyanobacteria during integrated membrane treatment and the subsequent potential release of algal metabolites (i.e., toxins) were also evaluated. The study shows that a tight NF membrane as a final stage of an IMS may be the best method for maximizing removal of extracellular cyanobacterial metabolites. An NF-IMS would be most suited to an area where cyanobacterial metabolites continuously occur. Lastly, the project developed several options for water utilities seeking an IMS for control of cyanobacteria and metabolites. These options span a range of complexity, capital and operating costs, and effort required to ensure process control.

The effect of common preoxidants used during water treatment on the integrity of cyanobacteria cells, and the subsequent release of toxic metabolites, odorous metabolites, and disinfection byproduct (DBP) precursors, was investigated in WRF project #4406, *Release of Intracellular Metabolites from Cyanobacteria During Oxidation Processes*. The digital flow cytometer provided a rapid method to obtain quantitative and qualitative information regarding cyanobacteria cell damage and lysis compared to conventional light microscopy. Results showed that cyanobacteria cell damage occurred without complete lysis or fragmentation of the cell membrane under the conditions tested. Results from this study showed that low oxidant exposures could result in the release of cyanobacteria metabolites. Depending on the cell concentration, oxidant exposure, and the magnitude of DOC release, sufficient intracellular organic material concentrations may be released resulting in impacts on regulatory compliance (THMs and HAAs) or consumer confidence (MIB and geosmin). With respect to microcystin-LR, utilities using chloramines ahead of any physical treatment barrier are at the greatest risk for releasing and accumulating microcystin-LR within the treatment process. Ozonation has shown the ability to release metabolites. However, ozone reacts rapidly with microcystin-LR and hydroxyl radicals, which can minimize the effect of the metabolite release via cell damage. Overall, physical removal of cells is recommended before the primary disinfection step in a water treatment process to avoid the release of these metabolites.

As most drinking water utilities still rely on conventional treatment, the objective of WRF project #4315, *Optimizing Conventional Treatment for the Removal of Cyanobacteria and Toxins* (2015), was to develop guidance for water utilities on the optimization of conventional treatment for the removal of cyanobacteria and metabolites while meeting all other water quality goals. Based on the results of the study, a set of recommendations for optimized operations during cyanobacteria challenges were developed:

- Do not use pre-chlorination for improved coagulation or reduced coagulant dosing during a cyanobacterial bloom unless comprehensive testing has identified a dose high enough to destroy released toxins. Do not apply pre-chlorination when cyanobacteria producing MIB or geosmin are present.
- Potassium permanganate dosing may be applied for the control of manganese and iron in the presence of *A. circinalis* and *M. aeruginosa*.
- Practice pH control to pH > 6 if this is not part of normal operations. This will reduce the risk of cell lysis and metabolite release during treatment.
- Optimize NOM removal using the criteria ΔC/C0 DOC, UV, and color < 0.05 and the cell removal should be optimized as well.
- While turbidity cannot be used as an indicator of the presence of cyanobacteria or cell concentration, use the decrease in settled water turbidity with coagulant dose as a surrogate for, or indicator of, cell removal if the initial turbidity is ≈10 NTU or above.
- If the presence of cyanobacteria results in increased coagulant demand to achieve improved settled water turbidity the application of a particulate settling aid, or even powdered activated carbon, may lead to improvements.
- Although removal of cyanobacteria through conventional coagulation can be very effective, 100% cell removal is unlikely in normal full-scale operations. In the event of high cell numbers entering the plant monitor for cell carryover and accumulation in clarifiers, this can lead to serious water quality problems if not rectified. Once captured in the sludge, cyanobacteria can remain viable and multiply over a period of at least 2–3 weeks. Simultaneously, within one day some cells in the sludge will lyse and release NOM and metabolites. Based on project #4315 findings, and to better understand the behavior of cyanobacteria, the dominant processes occurring in the sludge and supernatant, and the subsequent release of cyanobacterial metabolites, a follow-up project was initiated. The primary goal of *Management of Treatment Sludge Impacted by Cyanobacteria* (project #4523) was to provide operational guidance for the management of cyanobacterial sludge to ensure compliance with the regulations for cyanotoxins and to safeguard water quality. The main recommendation from this research was that a risk assessment and verification of existing treatment barriers be carried out assuming the “worst case scenario” of all cyanobacteria entering the plant, lysing, and resulting in dissolved metabolites returning to the head of the plant—possibly at a concentration 2–5 times higher than expected from a mass balance of the inlet concentrations. If the treatment barriers are insufficient to mitigate a potential challenge, sludge supernatant should be disposed of in an alternative manner for the duration of the bloom, unless regular monitoring of metabolites indicates significant reduction within the sludge treatment facility. This is particularly important in the presence of saxitoxins, which are recalcitrant to biological degradation.
Detection Methods

IN RESPONSE TO the increasing frequency of cyanobacterial blooms, greater awareness of toxic cyanobacteria, and new methods of detecting and monitoring cyanotoxins, robust analytical methods must be available to monitor for toxins and assess their significance. These methods either detect specific toxins or measure overall toxicity. There are several detection methods for cyanotoxins: high performance liquid chromatography (HPLC), gas chromatography coupled with mass spectrometry (GC/MS), liquid chromatography coupled with mass spectrometry or tandem mass spectrometry (LC/MS and LC/MS/MS), and enzyme-linked immunosorbent assay (ELISA). The toxicity assays include the neuroblastoma assay and the phosphatase inhibition (PIP) assays. The ELISA and PIP are currently commercially available. Depending on the cyanotoxin, one method may be preferable over another. More recently, molecular methods have been developed to identify the genes controlling toxin production.

Published in 2012, project #4212, Rapid Concentration and Detection of Microcystin and Other Cyanobacterial By-Products in Drinking Water, evaluated the use of surface enhanced Raman spectroscopy to concentrate and quantify cyanobacterial byproducts. The goal of the research was to develop a simple and economically feasible detection scheme for microcystin-LR and MIB that could be implemented in a water treatment facility. The study showed that drop coating decomposition Raman spectroscopy (DCDR) can be a powerful tool for qualitative and quantitative analysis of cyanobacterial by-products. The data suggests that DCDR can successfully identify microcystin-LR within a DOM matrix, produce reproducible spectra for samples up to 6 months old, quantity microcystin-LR in samples of 2–100 ng, and distinguish between similarly structured microcystin variants.

A report published in 2007, Determination and Significance of Emerging Algal Toxins (project #2789), co-sponsored with the CRCWQT, evaluated and developed methods available for detection of cyanotoxins. The study further developed methods for the detection of saxitoxin, anatoxin-a, and cylindrospermopsin. This included their detection by a single method; an LC/MS/MS method for detecting saxitoxin, anatoxin-a, and cylindrospermopsin in a single analytical run was successfully developed. (For low concentrations, pre-concentration using carbon-based solid phase extraction cartridges was required.) The neuroblastoma assay detected saxitoxin that was not detected by other methods. ELISA methods, including commercially available test kits, were also evaluated for detecting microcystin, with good results. PCR-based methods for the detection of toxic cyanobacteria were applied to a number of field samples, but it was found that determination of cyanobacterial toxicity using PCR-based assays needed further validation using a wider range of samples. Overall, the project suggests HPLC methods (and to a lesser extent, ELISA), can detect and quantify toxins present at low μg/L levels. Finally, an occurrence study detected microcystin in both U.S. and Australian raw water samples. Cylindrospermopsin was detected in Australian but not U.S. samples. Saxitoxin and anatoxin-a were not detected in any raw water samples in either country.

Several WRF projects researching the genetic basis for toxin production by cyanobacteria have been funded in the past few years. As most methods to detect toxins only work after the bloom has occurred, molecular tools, which can determine the potential of a population to produce toxins, may provide the best forecasting tool available for source water control strategies. Molecular technology enables spatial and temporal information on the distribution of toxic algae to be gathered rapidly with replication. These key advantages are not available when using microscopic analysis and provide an important augmentation to routine microscopy and toxin analysis when more detailed and rapid information about key toxic species is required.

A 2007 report, Development of Molecular Reporters for Monitoring Microcystis Activity and Toxicity (project #2818), identified regions of the Microcystis aeruginosa genome that could be used as molecular targets in the rapid identification of potentially toxic cyanobacterial blooms containing these cyanobacteria. This project developed a presence/absence approach (multiplex-polymerase chain reaction [PCR]), as well as a quantitative approach (using qPCR), which could be easily applied in field situations. To demonstrate that these molecular tools worked under field conditions, the researchers tested the probes during blooms of potentially toxic cyanobacteria Microcystis spp., which persisted in western Lake Erie in the United States during August 2002–2004, when microcystin concentrations exceeded the safety limit set by WHO. The presence of Microcystis spp. in water samples was confirmed through the multiplex PCR reaction using a combination of four primer sets. Quantification of Microcystis was achieved using the real-time PCR assay.

Early Detection of Cyanobacterial Toxins Using Genetic Methods (project #2881), developed a rapid genetic method to identify toxic cyanobacteria. A comprehensive literature review and industry questionnaire were used to identify and select a suitable platform technology for rapid genetic identification of toxic cyanobacteria. The project, co-sponsored with the CRCWQT, identified the genes likely to be involved in cylindrospermopsin production in C. raciborskii, and attempts were made to identify and sequence genes likely to be involved in the production of anatoxin-a. The project also developed a simple and rapid method for the preparation of cyanobacteria-containing water samples. Finally, a method was developed and tested successfully in the laboratory and the field to adapt conventional PCR assays for cylindrospermopsin-producing cyanobacteria to real-time PCR.

Project #4020, Methods for Measuring Toxins in Finished Water, investigated a range of biological methods as tools for source water and finished water toxicity measurements that may be suitable for detecting toxins in finished waters. Biological
assays have not been refined for application to drinking water. One of the key objectives of the project, co-sponsored with the Drinking Water Inspectorate (U.K.) and CRCWQT, is to define methods for quenching chlorine in finished water, as this is known to interfere with current toxicity screening methods (i.e., MicroTox® and CheckLight). Chemical quenchers were determined to be more suitable for use with bioassays than any of the physical methods of dechlorination evaluated. The appropriateness of the quencher, either sodium thiosulfate, sodium sulfite, ascorbic acid, or taurine, was determined by the assay. Surprisingly, a number of the bioassays tested were not adversely affected by chlorine, meaning that finished water samples can be tested in these formats without any quencher treatment. These assays included reticulocyte lysate assay for protein synthesis inhibitors, and the cell culture based assays utilizing either toxicity or genotoxicity endpoints. In addition to the effects of chlorine and the quenchers, the natural waters tested affected some assays. Thus, validation of bioassays using the waters they are intended to be used with should be included during assay establishment.

Because none of the aforementioned detection methods are standardized, WRF funded a research project with the EPA, *Criteria for Quality Control Protocols for Various Algal Toxin Methods* (project #2942), to develop quality assurance protocols for the quantitative analysis of microcystin, saxitoxin, cylindrospermopsin, and anatoxin-a present in water and of cyanobacterial extracts. The project report, which was published in 2010, recommended extraction methods, concentration methods, production of analytical standards, and preservation of water samples containing toxins of the various cyanotoxins.

**Source Water Control**

CYANOBACTERIAL BLOOMS OCCUR seasonally and are generally a result of over-enrichment by plant nutrients, particularly nitrogen and phosphorous. Human influences such as urbanization, increasing population, and agriculture contribute to the incidence of cyanobacterial blooms. As stated previously, not all cyanobacterial blooms cause the production of toxins. Cyanobacterial blooms may consist of strains not actively producing toxic metabolites, or producing several simultaneously; consequently, the toxicity of a bloom is difficult to establish. In addition, the use of copper sulfate in reservoirs to treat cyanobacterial blooms causes the cells to lyse and potentially release toxins. This uncertainty necessitates active source water control and monitoring of water quality for cyanobacterial toxins.

*Reservoir Management Strategies for the Control and Degradation of Algal Toxins* (project #2976), co-sponsored by CRCWQT, investigated cyanotoxin degradation in reservoirs by toxin-degrading organisms and developed reservoir management approaches for the control of toxin production using an ecological model. Reservoir hydrodynamics and growth of algal species were successfully simulated with the computer model. The timing and magnitude of blooms were similar for the field and the simulated data sets. The model was extended to include toxin production and degradation, which when applied to any reservoir would predict the risk of cyanobacterial toxins. The study also determined that utilities cannot rely on biodegradation to control microcystin, and cylindrospermopsin in drinking water reservoirs because toxins can be present in the water column without toxin-degrading bacteria being present. Cylindrospermopsin can persist for months in the water column, suggesting that biodegradation does not always occur. Additionally, screening with PCR for microcystin-degrading organisms revealed that these organisms were not always present in toxic blooms of *Microcystis aeruginosa*.

**Water Utility Guidance**

IN THE EARLY 1990s, several different countries, including Canada, Australia, and the United Kingdom, developed health guidance levels for microcystin in drinking water. In order to manage the potential hazard of cyanobacterial toxins, water suppliers needed knowledge not only of health risks, but also of the nature and causes of cyanobacterial blooms, the methods of monitoring and controlling toxins, the effectiveness of water treatment practices in removing toxins, and strategies for preventing and mitigating toxic bloom development.

Published in 1995, *Cyanobacterial (Blue-Green Algal) Toxins: A Resource Guide* (project #925) provides utilities with a comprehensive guide to addressing cyanotoxin concerns, including strategies for communicating with the public on potential risks of these toxins. The guide outlines several tiered approaches to managing, monitoring, and analyzing toxins. As an example, the monitoring plan, originally developed in Australia, introduces graduated response and action levels to increasing levels of toxic cyanobacteria in source waters. In Australia, the Alert Levels Framework developed in the 1990s as a monitoring and management action sequence provides a graduated response to the onset and progress of a cyanobacterial bloom. For instance, in the framework, Alert Level 1 (cyanobacterial biomass 2,000 cells/mL, 0.2 mm³/L biovolume, or 1 mg/L chlorophyll a) is the first step at which management action is taken.

Over the past 20 years, a number of organizations in several different countries have conducted research on managing cyanobacteria and the toxins they produce. The research was published in various papers, reports, and books, but consolidation of the knowledge was needed. The Global Water Research Coalition (GWRC), of which WRF is a founding member, addressed the need for a single, comprehensive resource for water suppliers worldwide with the project, *International Guidance Manual for the Management of Toxic Cyanobacteria* (project #3148). The manual includes perspectives from several countries on health effects, reservoir management, analytical methods, and treatment technologies to mitigate several species of cyanotoxins.
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