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Microscopes can do only so much when it comes to monitoring raw water and managing potential hazards such as algal blooms. Technological advances are opening new frontiers in understanding cyanobacteria, leading to improved monitoring and forecasting at levels of efficiency and effectiveness unheard of until now.

BY FRANCES BUERKENS, STEPHANIE A. SMITH, GREG FORD, AND HUNTER ADAMS

TACKLE TASTE AND ODOR WITH PROACTIVE WATER QUALITY MONITORING

WATER UTILITIES face increasing frequency and duration of taste-and-odor (T&O) events and cyanotoxin issues. Expedited analysis is critical for reservoir management, requiring utilities to revamp operations and conduct in-house testing. The COVID-19 pandemic may inspire permanent changes to some operations, requiring traditionally lab-based technicians to rely more on technologies that enable them to gather and analyze data remotely. Utilities must adapt to these dynamic environmental and social conditions, leading many to explore how technology can facilitate affordable, scalable, repeatable monitoring programs.

Many biological monitoring programs still depend on the same technology that Dutch scientist Antonie van Leeuwenhoek used in 1676 to discover the first bacteria observed by humankind: the microscope. Although microscopes are a key fixture in every microbiology lab, their development has slowed because optical limits have been largely reached. Advancement potential exists, with higher magnification to better observe individual cells, but this is unlikely to change how utilities monitor raw water. Utilities can look to new technologies to bring a broad environmental picture into focus.

PHOTOGRAPH: YSI XYLEM

Multiparameter sondes with a dynamic range of smart sensors are available to collect water quality data day and night, providing early detection for algal blooms and bloom dynamics.

COMPREHENSIVE MONITORING

From the field to the lab, a water utility's goal is to seek information about one variable: change. Dynamic environmental conditions require responsive tools to reveal potential problems. Cyanobacteria and algae populations can bloom within hours. Blooms that remain unchecked can attract unwanted attention, undermining confidence in public water systems. However, there is no single solution to track change. Biology is elusive, and complex problems evade simple answers. Utilities can respond in two ways: (1) increase sampling frequency and number of locations, and (2) integrate multiple tools that expand an analyst's field of view.

It's impossible to detect change fast enough to make effective decisions without ample data across multiple locations and time scales. If data are limited in frequency or location, accurately tracking cyanobacteria and algae populations turns into a wild goose chase. Cyanobacteria were the first group of living organisms on Earth. Outlasting dinosaurs gives credence to their evolutionary capabilities.

Cyanobacteria benefit from diurnal variations, enabling them to outcompete algae. Propelled by buoyant cells, cyanobacteria rise to the surface at night to absorb more light and sink during the day to enhance nutrient absorption. Because we can't assume that cyanobacteria are evenly distributed across a body of water, monitoring programs must encompass a broad spatial and temporal view that accounts for changes throughout day to night, spring to summer, and pre- to post-treatment. A 1-mL sample—concentrated or not—analyzed once a week can't provide a comprehensive view.

NEW TECHNOLOGIES

Increasing sampling frequency and locations is often difficult, as labs are frequently understaffed and overloaded. Fortunately, remarkable technological progress has taken place in recent years, making it possible to expand monitoring programs with a limited team. Three relatively new technologies—multiparameter sondes, semiautomated flow-imaging microscopes, and DNA-based assays—can be integrated from the field to the lab to form the foundation of a comprehensive screening program for cyanobacteria and address concerns about the production of T&O compounds as well as toxins.

Collecting and Understanding Data. Used in the field, multiparameter sondes, such as the EXO series from YSI Xylem (www.ysi.com), are remotely deployed, collecting data day and night. Sondes serve as "first responders," providing early detection for blooms and bloom dynamics. By collecting statistically significant data sets, sondes establish a baseline to determine what's normal for a particular location. Every location has a unique profile, much like a fingerprint; sondes show what that fingerprint looks like so when something changes—such as temperature, pH, dissolved oxygen (DO), chlorophyll, or phycocyanin levels—utilities can determine if that change is a precondition to a bloom.

Chlorophyll and phycocyanin monitoring provide early indication of a potential bloom, but a thorough understanding of bloom patterns is gained by adding additional parameters. Monitoring temperature reveals what temperature range supports harmful algal bloom (HAB) formation in each reservoir. Water treatment plant operators and managers should learn how temperature aligns with seasonal, spatial, and temporal bloom patterns. Reservoirs in temperate climates may host spring or fall blooms that result from different populations of algae and cyanobacteria.

DO refers to the equilibrium in which gases naturally dissolve into water. DO patterns change with HABs. During the early and peak growth phases of an HAB, DO can increase significantly in the vicinity of the bloom as a result of exceedingly high photosynthetic activity. More oxygen is generated than can be consumed by either the cyanobacteria or other organisms, leading to supersaturation in which DO levels exceed 100 percent. As blooms fade, algae become food for bacteria and other organisms that consume oxygen, at which point DO levels can drop precipitously. The result is hypoxia.

It's a common misperception that cyanotoxins kill fish in surface water reservoirs, but the culprit is most often hypoxia. The size of the bloom relative to the size of the water body and the proximity of a DO sensor to the bloom or oxygen-consuming bacteria affect one's ability to observe these patterns for managing a reservoir. In addition, DO monitoring aids in understanding the efficacy of aerators used to prevent stratification.

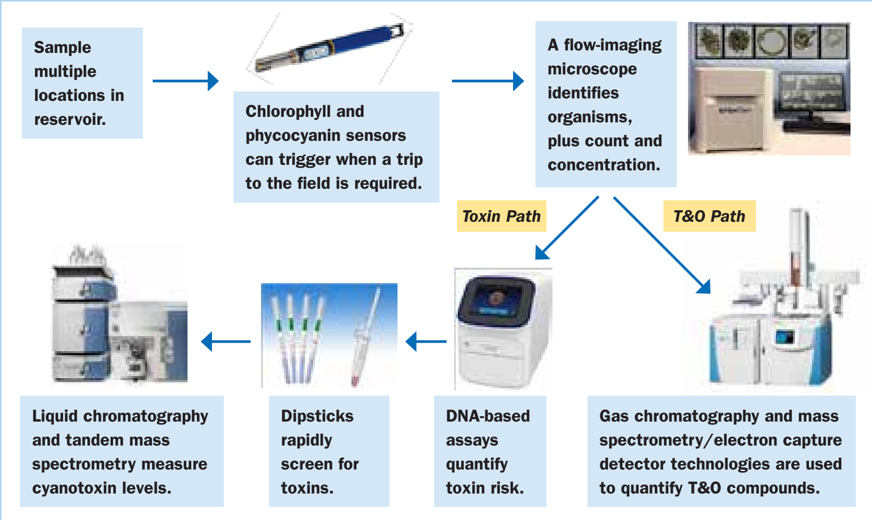
Fluorescence-based pigment detection is another powerful tool to monitor cyanobacteria and algae populations from afar. Two individual pigments, chlorophyll and phycocyanin, warn whether the growth might be an algal bloom or a cyano-HAB. Chlorophylls a and b are found in all eukaryotic algae. Cyanobacteria contain chlorophyll a and phycocyanin. Every reservoir has a unique baseline, ideally monitored in relative fluorescence units. Deviations from that baseline can alert analysts of a bloom in its early stages. Phycocyanin levels make the distinction of a cyanoHAB possible when both pigments are monitored. When used with a logging instrument and telemetry, pigment detection can reduce trips to the field, optimizing when to collect samples or perform other analyses.

Simplifying Identification. Once a sonde has validated that a bloom may be forming and a trip to the field is deemed essential, the second line of defense is to identify what organisms are in the raw water and quantify how many are present. A semiautomated flow-imaging microscope, such as the Flow-Cam Cyano from Yokogawa Fluid Imaging Technologies (www.fluidimaging.com), identifies and enumerates cyanobacteria and nuisance algae. Although the traditional microscope is a superior tool for species-level identification, most utilities limit identification to the genus level

Rather than waiting for a bloom, proactive utilities can spot treatments and address a problem in its nascent stages.

Integrated Toolkit for T&O and Toxin Monitoring Pathways

Relatively new technologies can be integrated to form a screening program for cyanobacteria and algae as well as address concerns about T&O compounds and toxins.



or functional group. The FlowCam Cyano speeds up this time-consuming process by presorting data into three functional groups: cyanobacteria, diatoms and other algae, and detritus and decomposing organisms. Technicians sort the remaining data by morphology through the use of image-recognition software, taking care to identify the most prolific genera and grouping the remaining low counts into more general categories. This strategy deviates from traditional approaches, but it's a simplified methodology with a turnaround time of five to 30 minutes in most samples, including sample preparation and data collection.

Species-level identification requires an investment in time and considerable taxonomic skill, preventing many utilities from taking this approach. Some utilities have developed long-term species-level data sets by partnering with expert taxonomists. These incredible resources are difficult to seamlessly merge with a flow-imaging microscope because the data acquisition and data analysis methods are different. Correlating microscope results with a flow-imaging system requires patience, but the result is a statistically significant data set with a same-day turnaround time.

This topic isn't straightforward and is sometimes considered controversial by utilities that appreciate the value of speciation. However, treatment decisions are rarely affected by determining the exact species. The move from species- to genus-level identification with a flow-imaging microscope offers speed and repeatability in a time-constrained environment.

Samples identified to species level using traditional microscopy are rarely analyzed more than once a week and often have a turnaround time of three days to three months, at which point conditions have changed and the data become useful only as a historical snapshot. Although microscopic records must be transcribed and digitized, a flow-imaging microscope automatically saves a digital image of all organisms in the sample, along with a comma-separated values report of the count, concentration, and size of the organisms. Reports are customized by the operator to highlight populations more likely to cause a problem, allowing technicians to spot organisms posing higher risk in each day's samples.

What can take hours by microscope takes minutes with a flow-imaging microscope, creating an opportunity for utilities to commit to a treatment plan within a matter of hours instead of days or weeks. The transition to decreasing turnaround time, increasing sampling frequency, and increasing sample locations provides the statistically significant understanding utilities need to make qualified treatment decisions.

The number of cyanobacteria and algae genera can feel overwhelming in light of the discovery of new species and reclassification of known species. Fortunately, there's a relatively short list of nuisance organisms, aptly named the Dirty Dozen. The organisms that wreak havoc are often repeat offenders, with *Dolichospermum* (aka *Anabaena*), *Microcystis*, and *Aphanizomenon* earning a place at the top of the global "most-wanted" list. Although cyanobacteria regularly make headlines, green algae, golden algae, and diatoms create T&O trouble as well. Almost any type of algae can induce a T&O event at a high enough density, so it's critical to know if any one genus is flourishing. Upon detection, spot treatment in reservoirs can address problems as they arise. This methodology ensures that algae and cyanobacteria don't form significant blooms, dodging a problem that's difficult and expensive to treat.

Detection and Quantification. The traditional metric for measuring cyanobacteria concentration is a cell count. Sondes track an increase or decrease in concentration, and a flow-imaging microscope determines the cell count for each genus. A molecular-based technology, such as the CyanoDTec Total Cyanobacteria Assay from Phytoxigene (www.phytoxigene.com), measures the 16S rRNA gene common to all cyanobacteria, thereby quantitatively measuring the number of cyanobacteria present. Because of variations across genera, there's no correlation between cell count and gene copy numbers; however, increases in either are indicative of bloom growth. Should a *Microcystis* bloom take place, counting cells per milliliter or running the Total Cyano Assay will indicate the concentration of the bloom, but it won't confirm toxicity. The scientific community hasn't yet determined what triggers a bloom to become toxic, but there are tools available to rapidly predict whether toxin production is possible.

Cyanotoxins are produced by many strains of cyanobacteria spanning multiple genera. However, because toxicity isn't uniform among strains, conventional bacteriological classification methods are unable to accurately predict toxicity. Analytical methods for detecting toxins often take days to perform and aren't predictive. Thanks to recent advances in understanding the biosynthetic pathways of toxin production, analysts can detect target genes that are critical to the production of cyanobacterial toxins in environmental samples.

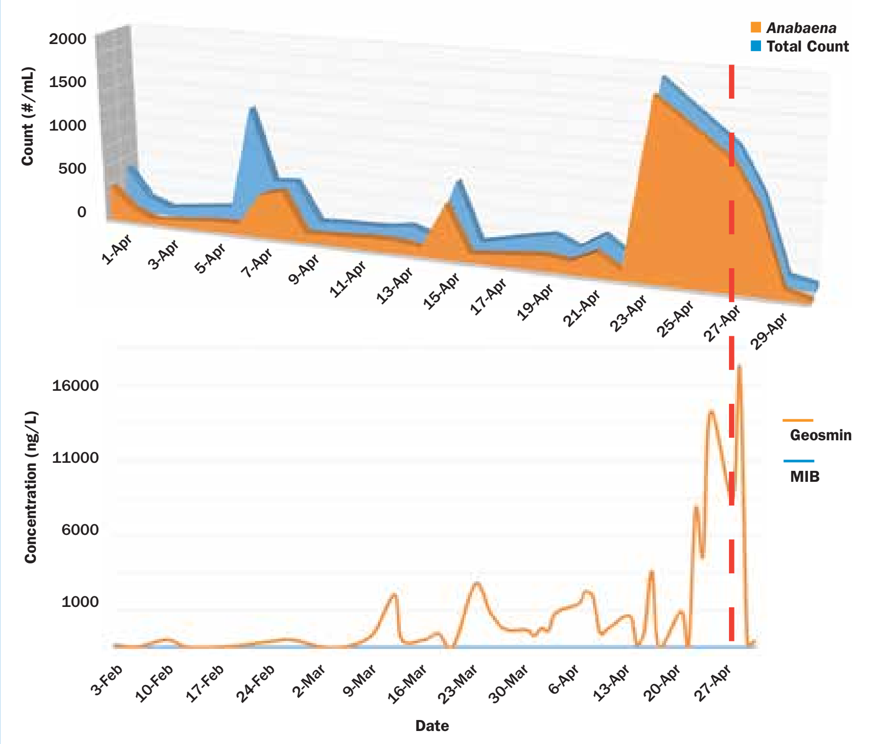
Many cyanobacteria can produce toxins, and many can produce more than one type of toxin. However, not all algal blooms are toxic. Because a DNA-based assay such as CyanoDTec detects and quantifies the presence of cyanobacteria and their toxin-producing genes in environmental samples, it can be used to answer two questions: When should a water body be tested for toxins, and which specific toxin should be tested? In less than three hours, laboratory technicians can know if cyanobacteria are present and which toxin poses a risk. Technicians can identify and quantify the presence of total cyanobacteria, along with four genes responsible for producing toxins: microcystin, nodularin, cylindrospermopsin, and saxitoxin. The state of Ohio recently ran paired samples of the CyanoDTec Toxin Gene Assay and microcystin measurement by enzyme-linked immunosorbent assay and had a 100 percent correlation of gene detection, with toxin measurement below the US Environmental Protection Agency's threshold of 1.6 μg/L.

Treatment solutions are like goalies—they're a key team member but should be the last line of defense against 2-methylisoborneol (MIB), geosmin, and cyanotoxins. Treatments should be used strategically as a last resort; otherwise, utilities could inadvertently inflate a problem. It's important for operators to know if they're treating potentially toxic cyanobacteria. Identifying organisms to genus level can be critical in determining whether a human health event could be at hand, and toxin gene measurement quantifies the associated risk level. Adsorptive treatment, such as powdered activated carbon, can only do so much for a toxin event. Copper sulfate will lyse cells; if those cells contain toxins, those toxins become considerably more difficult to remove from treated water.

T&O management follows a similar pattern. Lysing a small number of cells can prevent a large T&O event, and preventing further cyanobacterial growth minimizes the event's scale. Rather than waiting for a bloom, proactive utilities can spot treatments and address a problem in its nascent stages. If cells from a bloom lyse, MIB and geosmin can linger for days or weeks.

T&O in Lake Arrowhead Near Wichita Falls, Texas

Algae counts measured by a flow-imaging microscope in late April 2020 revealed elevated levels of *Anabaena* (top), which often correlates with a geosmin spike (bottom).



A BRIGHT FUTURE

Water utilities depend on scientists and technicians to interpret results and determine how to mitigate problems. Although technological advancements have transformed monitoring methods, these approaches fail to produce simple answers.

Despite this challenge, the future is bright. Scientists have a culture of sharing information, and talented leaders have turned a weakness into an opportunity for advancement. A follow-up article in *Opflow*'s November issue will feature thought leaders across the United States who have proved through critical thinking, strategic scientific analysis, and hard work that cyanobacteria and algal blooms can be managed effectively and affordably.