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Eric Duderstadt is a water biologist/chemist
with Water Systems Engineering Inc.
(www.h2osystems.com), Ottawa, Kan.

Customize Biological Testing for Best Results

Although numerous tests are available for analyzing bacteria, they vary greatly in price and time involved. The best approach is one that accounts for the greatest number of bacteria in an acceptable time frame at a reasonable cost. BY ERIC DUDERSTADT

FOUL ODORS, production loss, corrosion, and overall water quality decline can be caused by microbial populations. Assessing biological activity within a water system helps determine water's overall cleanliness and assess system operation. Identifying microorganisms is useful in diagnosing problems, determining the most effective treatments, and guiding routine maintenance operations. Periodic bacterial monitoring can also help lower treatment costs, such as maintaining chlorine residuals, and reduce costly unscheduled maintenance.

Several methods for analyzing bacteria are currently available, including adenosine triphosphate (ATP) analysis. More conventional tests include coliform testing, the most probable number (MPN) technique, total cell or particle counts, DNA and RNA testing, polymerase chain reaction (PCR), variations of the heterotrophic plate count (HPC), and numerous tests for specific organisms.

A biologist examines colonies of heterotrophic plate count bacteria after 48 hours of incubation on growth agar.

QUANTIFICATION METHODS

Advances in science and technology during the last few decades have allowed for more accurate bacteria counts in water.

ATP. ATP analysis is a simple chemical test that can quickly and accurately monitor microorganisms in a water sample. ATP, a molecule often referred to as "energy currency" because of its universal use as an energy source in cells, is manufactured during cellular respiration and can be found in every living organism—from humans to single-cell bacteria.

The test exposes microorganisms to enzymes that catalyze a reaction, converting ATP into light energy. Each molecule of ATP consumed in the reaction produces one photon of light, the output of which is measured by luminometers and compared with a standard to calculate the amount of biovolume. However, diversity among waterborne microorganisms is vast. Differences in metabolic processes, shapes, sizes, food sources, and physiological states can influence ATP concentrations. Nonetheless, recent studies have revealed information regarding

A biologist transfers a membrane filter to its growth support medium for incubating coliform bacteria.

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cellular ATP concentrations in individual bacteria. Average ATP levels help estimate bacterial count in samples.

ATP analysis is a reliable test because it accounts for all living organisms present, isn't influenced by inorganic particulates, provides accurate bacteria counts, and detects bacteria considered to be unculturable. Given these advantages and considering the test's quick results, low costs, and reproducibility, ATP analysis is an effective tool in monitoring bacteria in water.

HPCs. Another common way to analyze bacterial presence, HPCs estimate the number of heterotrophic bacteria (organisms that require organic compounds for nourishment) in a sample. Although the term HPC first appeared in the 16th edition (1985) of *Standard Methods*, counting bacteria on nutrient agar plates dates back to the late 1800s.

Three common HPC methods—spread plate, pour plate, and membrane filtration—introduce a given sample quantity to a nonselective media with the aim of culturing aerobic bacteria. Bacteria appear on the media as colonies, which are counted to determine the number of bacteria present. HPCs are useful because they don't count dead organisms or inanimate particulates. However, one colony may develop from a single cell or numerous cells, and cell clumping in colonies is random, making accurate bacterial counts difficult.

Perhaps the HPC method's biggest disadvantage is that only a small percentage of bacteria found in water systems is culturable using common laboratory media, with the uncultured fraction including diverse organisms only distantly related to cultured ones. Therefore, culture-independent methods are essential to understanding the genetic diversity, population structure, and ecological roles of most microorganisms.

Other Methods. Other quantifying methods, such as particle counts, are also available but may fail to distinguish dead organisms, living organisms, and other particulates present, and still other methods can be time consuming and expensive.

Coliforms. Coliform testing is widely accepted for determining drinking water's sanitary quality. Coliforms are a group of closely related bacteria that behave much like a variety of other bacteria, parasites, and viruses known to be harmful if consumed in drinking water. Though coliforms also occur naturally in aquatic environments, and most aren't harmful to humans, it's their similarity to microorganisms that have earned them the role of indicator organism. However, the presence of coliforms only *suggests* the potential presence of more problematic organisms; they don't confirm it. Conversely, the absence of coliforms doesn't confirm a water sample is free of harmful microorganisms.

IDENTIFICATION METHODS

Many methods are available to identify individual bacteria or bacteria classes. Two types of bacteria are of particular interest in potable water systems: nuisance organisms and pathogenic or opportunistic pathogenic organisms.

Nuisance organisms—including those that influence taste, odor, color, and

A biologist prepares HPC pour plates by adding liquefied agar to 1 mL samples in petri dishes, which then get incubated for 48 hours.

turbidity—can reduce water's aesthetic quality. Monitoring for these organisms is useful because they're a health threat, can damage system infrastructure, and cause increased maintenance and treatment costs.

Pathogenic or opportunistic pathogenic organisms pose health risks if consumed, especially if consumed by individuals with weakened immune systems. Although coliforms fall within this group, the group isn't limited to coliforms. In fact coliforms share this category with thousands of other species, including *Legionella*, *Salmonella*, and *Streptococcus*.

Tests that identify specific bacteria include PCR, DNA sequencing, phenotype microarrays, and nutrient characterization assays. Some nuisance organisms, such as iron-oxidizing bacteria, can also be identified via microscopy. Identifying these specific organisms isn't always necessary but can be helpful in locating problems in a system and setting maintenance priorities. Removing these organisms can also prevent compliance failures.

A COMPREHENSIVE APPROACH

Although the aforementioned methods have advantages and disadvantages, each provides insight into bacterial loads in water. Using several tests in combination provides a more complete picture of bacterial fouling.

Knowing which tests offer the greatest benefits often requires evaluating the system. System design, environmental influences, and knowledge of previous problems are variables that can help determine which tests are most valuable. However, the best approach is the one that accounts for the most bacteria in an acceptable timeframe at a reasonable cost. Much like a census, a well-structured assessment will identify as many groups of bacteria as possible as well as probable group locations and size. This information allows for more accurate treatment and more efficient system operation.

COVERING ALL BASES

A thorough bacterial assessment should answer the following questions.

How Many? An exact count isn't always necessary, but an estimate of the total number of bacteria present helps determine how established communities are and the level of need for cleaning or disinfection. ATP and HPC tests are appropriate.

What Kinds? Identifying the major players is key. Simple staining procedures reveal whether organisms are gram negative or gram positive. It's valuable to know if nuisance organisms—iron-oxidizing and sulfate-reducing bacteria, for example—are present and what threats they pose. Testing for iron-oxidizing and sulfate-reducing bacteria is fairly quick and inexpensive using simple tests designed to measure the reaction of populations to specific nutrients.

Is It Safe? Total coliform and *E. coli* coliform testing is the most widely used method for determining safety from a bacterial aspect. However, numerous other organisms can cause illness. Evaluating the conditions these organisms need provides some indication of the likelihood of their presence. For example, biofilms—habitats created by bacteria for protection and nutrient capture—can harbor problematic organisms such as coliforms. Several methods are available for identifying these specific bacteria, but those that employ simple single-color redox chemistry to detect reactions within bacterial cells are easy to use and provide quick results.

Is Action Needed? A series of tests will identify the water's overall condition as well as problem areas that may need attention. Data generated by laboratory testing should be interpreted to identify problems and determine the most effective treatments. Results can also guide sustainable operations and reduce treatment and unscheduled maintenance costs.

RESOURCES

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