

Fecal Indicator Bacteria

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Background

Fecal indicator bacteria (FIB) assays are used as surrogate measures of the sanitary quality of surface waters and industrial discharges. Most states have recreational water quality criteria (RWQC) that specify limits for FIB, with total coliform, fecal coliform, *Escherichia coli* (*E. coli*), and enterococci classified as FIB. Limits vary depending on the designated use of the water body, time of year, and other local factors. To ensure that treated pulp and paper mill effluents do not pose a risk to human health and to protect recreational use (e.g., swimming, fishing, boating) in receiving waters, National Pollutant Discharge Elimination System (NPDES) permits may include limits or monitoring requirements for FIB. NPDES permits specify the FIB endpoint to be measured, the frequency and timing of testing (e.g., year-round or seasonal monitoring), and the acceptable analytical test methods.



Historically, fecal coliform was the most common FIB monitoring endpoint in NPDES permits. However, in response to EPA's RWQC recommendations, there has been a shift to monitoring for *E. coli* in freshwaters and enterococci in marine/estuarine waters because results from these assays provide a more robust association with illness rates in epidemiological studies than other FIB endpoints. As states update their RWQC criteria to be consistent with EPA's recommendations, mills that previously had fecal coliform limits and/or monitoring requirements in their NPDES permits are more likely to see *E. coli* and enterococci limits and/or monitoring requirements in the future. Despite this change, the Food and Drug Administration (FDA) continues to require fecal coliform monitoring in shellfish harvest zones, and fecal coliform may also be required if the receiving water has a Total Maximum Daily Load (TMDL) for fecal coliform.

Analytical Methods

There are three main types of FIB tests, including multiple tube fermentation, membrane filtration, and most probable number (MPN) enzyme substrate tests. The latter two tests are most commonly used for mill effluents. Membrane filtration tests involve filtering the sample, placing the filter on selective media and incubating for a specified time interval, and directly counting the colonies that meet specific colony characteristics (e.g., color, size). The MPN enzyme substrate tests (e.g., Colilert[®],

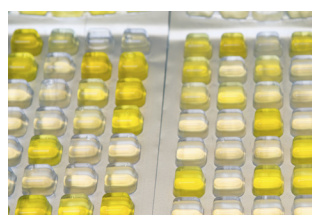


Photo: Colilert quantitray

Enterolert[®]) are based on a reaction between certain bacterial strains and proprietary substrates in a multiple-welled tray that cause either a color change or fluorescence. Incubation temperatures enable total

coliform and *E. coli* (35°C) or fecal coliform (44.5°C) to be determined using the Colilert[®] test, or enterococci (41°C) using the Enterolert[®] test. Results of membrane filtration tests and MPN substrate tests are reported as colony forming units per 100 ml (CFU/100ml) and the most probable number per 100 ml (MPN/100ml), respectively. Enzyme substrate tests are advantageous because they are comparatively easy, less costly, and generate results more rapidly than membrane filtration or multiple tube fermentation methods. Regardless of test method, it is recommended that multiple effluent dilutions are used to ensure that results are within the desired range for the test.

Implications for Mills

Environmental strains of bacteria not associated with human fecal sources can contribute to elevated FIB or, in some cases, can contribute to false positives. These include fecal contamination from wildlife or bacterial strains associated with wood decay. Because the fecal coliform test is a measure of several thermotolerant (i.e., heat tolerant) bacterial strains, the *E. coli* test method is the preferred endpoint for mill effluents. This is largely because bacterial strains associated with wood decay (e.g., *Klebsiella*) are measured in the fecal coliform test and can contribute to false positives with the *E. coli* test. Generally, most mills that are able to meet fecal coliform limits will also be able to meet limits for *E. coli*. However, NCASI studies indicate that few mills can consistently meet RWQC for enterococci because *Enterococcus* species associated with plant sources (e.g., *Enterococcus casseliflavus*) are frequently detected in mill wastewaters and woodyard runoff. Due to these challenges, some mills may need to use molecular source tracking methods alongside traditional FIB methods to assess whether bacteria are due to human or non-human sources. This includes species confirmation testing and/or tests for human or animal genetic markers to determine if elevated FIB are due to human or non-human sources.



Photo: *Escherichia coli* (*E. coli*)

NCASI continues to monitor the development and use of bacterial indicators and their utility in pulp and paper mill effluents and provides ongoing support to members facing challenges with bacteria monitoring.

For more information contact:

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