

Wastewater *E. coli* Methods: A Review

Outline

- Regulatory
- Noted Issues
- Sampling
- Methods
- Evaluation of Colonies

Regulatory

- 30 TAC Chap. 309; Domestic Eff. Limits
 - Subchapter A; Rule 309.3
- (4) Except as provided herein, disinfection of domestic wastewater which is discharged by means of land disposal or evaporation pond shall be reviewed on a case-by-case basis to determine the need for disinfection. All effluent discharged to land to which the public has access must be disinfected and if the effluent is to be transferred to a holding pond or tank, the effluent shall be rechlorinated to a trace chlorine residual at the point of irrigation application. All effluent discharged to land via a subsurface area drip dispersal system to which there is a potential for public contact shall be disinfected and shall comply with an *Escherichia coli* (*E. coli*) bacteria effluent limitation of 126 colony forming units per 100 milliliters of water or a fecal coliform effluent limitation of 200 colony forming units per 100 milliliters water, per grab sample, in accordance with paragraph (1) of this subsection.

Issues With The Sewage

- Influent Strength
- Industrial Users, Car Washes, Restaurants Can Produce High Strength, Toxic, or problematic Wastewater
- Degree of Inflow/Infiltration
- Lack of Collection System Maintenance

Issues At The WWTP

- MLSS too High in Aeration Basin
- Dissolved Oxygen Level Too low in the Aeration Basin
- Bulking Sludge in The Clarifier (Filamentous Organisms)
- Poor Attention to The WWTP Overall

Additional Issues At The WWTP

- Clarifier Issues: Weirs, Hydraulics
- Scum, Grease
- Turbid Effluent
- High Chlorine Demand; BOD/TOC Load
- Must Have 20 Min. Detention Time at Actual Peak Flow
- No Excess Sludge Settled in Chamber

Too Much Solids!



Sampling

- Samples Should be Analyzed Immediately
- Preferably Within 2 Hours of Collection
- Max-Transport Time is 6 Hours
- Processed Within 2 Hours of Receipt at Lab

Sampling Techniques

- Where to Collect It? (Needs To Represent What Hits the Creek or Designated Outfall).
- How to Collect It!
- How Much to Collect?
- Process After Collection

Methods

- EPA Method 1603: *Escherichia coli* (*E. coli*) in Water by Membrane Filtration Using Modified membrane-Thermotolerant *Escherichia coli* Agar (Modified mTEC)
- HACH 10029 m-ColiBlue24
- Enzyme Substrate Coliform Test, the Colilert® System with Quanti-Tray® (IDEXX)
- Others?

Laboratory Techniques

- Complete Understanding of Methodology Utilized
- Well Trained in Method
- It's All About Sterile and Performance Technique
- Proper Apparatus

Analysis Steps

- Some Effluents Often Can Not be Cultured at Full-Strength (100 mL.)
- Serial Dilution Techniques Using Buffered Dilution Waters (DI-Water)
- There Are Several Available Tables and Graphs Illustrating Volumes to Be Filtered for Chosen Method
- May Need to Set-Up Various Dilutions

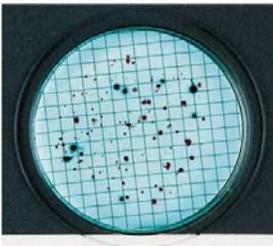
Results

- What Does the Method Say?
- Are There Specific Colony Colors or Agar Changes?
- Counting Steps or Enumeration Readings on IDEXX Method
- What About MPN?
- Do You Need a “Positive” Colony (Sample)?

Results Cont'd.

- Visually Determine Colony Counts on Membrane Filters
- Use Stereo Microscope or Colony Counting Magnifier Unit

M-ColiBlue24



- Colony appearance:
 - * Red or Blue Colony- Total Coliform Positive
 - * Clear or White Colony Total Coliform Negative
 - * Blue colony is specifically an E. coli Positive
 - * Non-Blue- E. coli Negative

M-ColiBlue24

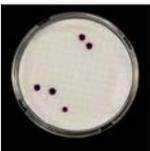
- HOWEVER! Insure You Read m-CB24 Trouble-Shooting Guide from HACH
- This Provides The Details on Expected Reactions of Various Microorganisms with m-ColiBlue24 That can Grow on The Media That Are Not of The *coliform Family*
- In Other Words: There Are False-Positives
- Follow Through With Described Oxidase Testing of The Petri Dish Filter to Confirm *E. coli*

IDEXX



- Very Specific Counting Technique (UV)
- Follow Through With Directions
- There Are Documented Descriptions on False-Positives
- Insure A "Positive" Sleeve is Compared Too

EPA Method 1603



- Modified m-TEC Agar
- The need for this change can best be understood by comparing results obtained using a chromogenic substrate as opposed to those obtained using an urea/phenol substrate.

Summary

- So Important To Understand What is Being Done at That Specific WWTP
- What Are The Technical Capabilities of The Staff and Its Laboratory?
- Technique Driven, Proper Training on All Aspects of Bacterial Testing for MDR Compliance
- Too Many Errors, Not Proper Method Utilized and Such

Questions?

Thank You!
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