## Effect of holding time on *E.coli* Densities in Wastewater Samples

Nicki Sava-Schafer, Debmalya (Deb) Bhattacharyya, Mark Citriglia Northeast Ohio Regional Sewer District



### Outline

What we (NEORSD) do? Bacteria Hold time for regulatory programs **NEORSD** problem statement **Recent studies performed** Sample collection **NEORSD** data Sample storage Summary Sample preparation



## NORTH EAST OHIO REGIONAL SEWER DISTRICT NEORSD (http://www.neorsd.org)

- The District was formed in **1972** and is responsible for **WWTP** and **interceptor sewers** in the greater **Cleveland Metropolitan Area**. This service area encompasses the **City of Cleveland** and all or portions of **61 suburban municipalities** in Cuyahoga, Summit, Lake and Lorain Counties and includes a diversified group of manufacturing and processing industries
  - Wastewater Treatment Plants (WWTPs): 3 WWTP
  - Interceptor Sewers: Maintains over 200 miles of large interceptor sewers.
  - Combined Sewer Overflow Control: Maintains CSO throughout the Greater Cleveland area.
  - Industrial Waste Control in the area
  - Other Areas: Watershed protection and planning, small streams and tributaries are properly maintained

#### **NEORSD Wastewater Treatment Plants**



**Southerly WWTP** 



**Easterly WWTP** 



Westerly WWTP



# **Analytical Services**



- Providing the three WWTPs, WQIS with analytical results of daily wastewater samples for parameters required by the NPDES permit, Whole Effluent Toxicity (WET), 503 sludge regulations, other operational needs for surcharge and enforcement purposes.
- Performing analysis for designated lake and river site samples.

### **NEORSD Laboratory Problem statement**

 Due to a CSO Consent Decree form the USEPA, and various NEORSD water quality projects dealing with the assessment of bacteria and nutrients has forced the NEORSD laboratory to be creative with staffing, automation, methodology and leveraging holding times to meet the demands and workload of these projects.

## **NEORSD Consent Decree**

#### CSO Consent Decree with the USDOJ, USEPA and Ohio EPA (2011)

- Reduce Combined Sewer Overflow and plant bypass events by 89%
  - Reduction from 4.5 billion gallons to .49 billion gallons in 25 years
- Infrastructure Changes (Construction)
  - Treatment plant enhancements including Chemically Enhanced High Rate Treatment (CEHRT) with simultaneous disinfection
  - Gray infrastructure Tunnel Projects
  - Green Infrastructure projects (Stormwater Control Measures)
  - Illicit discharge detection and elimination
- Monitoring

- Understanding our 25-year project plan to protect Lake Erie
- Pre and post construction monitoring for bacteria, nutrients, and biological assessments



## **Combined sewer overflow (CSO)**





## **NEORSD Project Summary**

#### Chemically Enhanced High Rate Treatment (CEHRT)

- Construct a CEHRT facility at each of the 3 NEORSD WWTPs
- Scope of Work
  - Wastewater characterization for dry and wet weather (10 parameters including bacteria E. coli)
  - Jar testing to determine chemical treatment and disinfection
  - Operation of a pilot plant to prove concept
  - Construction and post construction monitoring
- Project Sampling Plan
  - Collect influent and effluent samples for each event up to 12 hours
  - Perform jar testing for disinfection 36 72 samples per event
  - The validation and testing will occur over 20 wet weather events
  - Samples = 4600 for bacteria, and 1600 for conventional parameters
  - Sampling can occur at anytime of the day or night



**Chemically Enhanced Primary Treatment** (CEPT) and High Rate Disinfection (HRD)

## **NEORSD Project Summary**

- WWTP chlorination process optimization
  - Sample collection for bacteria at 1 hour intervals to assist with process optimization
- CSO permit requirements for monitoring of bacteria
- Local sanitary sewer evaluation study
  - Identify bacteria loads during dry and wet weather events at local stream
- Integrated planning initiatives
  - Determine CSO impacts to Lake Erie and tributaries during wet weather events
- Illicit discharge determination and elimination (IDDE)
  - Dry and wet weather events

# **Holding Time**



- 40 CFR 136.3(e) Table II: Required Containers, Preservation Techniques, and Holding Times (notes)
  - <sup>22</sup> Sample analysis should begin as soon as possible after receipt; sample incubation must be started **no later than 8 hours** from time of collection.
  - <sup>23</sup> For fecal coliform samples for sewage sludge (biosolids) only, the holding time is extended to 24 hours for the following sample types using either EPA Method 1680 (LTB-EC) or 1681 (A-1): Class A composted, Class B aerobically digested, and Class B anaerobically digested.
- 40 CFR 141.21
  - The time from sample collection to initiation of analysis may not exceed 30 hours. Systems are encouraged but not required to hold samples below 10 deg. C during transit.

# **NEORSD Laboratory Challenges**

- The short holding time associated with bacterial analyses coupled with large sample loads stretches the ability to effectively manage a laboratory
- Challenges
  - Staffing issues
    - On-call schedule
    - Off-shift work and overtime
  - Rain events do not always occur during normal business hours
  - Flow based sampling events
  - Difficult to meet holding-times for large sampling events
  - Media validation, autoclaving and supplies

## **Project Management Concerns**

- PM Concerns
  - Want method compliant data for large sampling events
  - Bacteria results will not be representative and biased high or low
  - Issues with an MPN method (Colilert<sup>®</sup>) and historical cfu/100ml data from membrane filtration (MF)
  - Using coolers and ice to store samples until the lab was open
- Laboratory's Response
  - Perform a validation study on the Colilert<sup>®</sup> method vs MF
  - Perform a holding time study for *E. coli* using various sample types
    - Raw Influent High bacteria, solids and organics load
    - Primary Effluent High bacterial load, moderate solids and organics load
    - Secondary Effluent Moderate bacteria, and low solids and organics
    - Treated Effluent disinfected Low bacteria, solids and organic

## **Laboratory Goals**

- Perform as much analytical testing as possible during normal working hours
  - Monday Friday 7:00am 5:30pm
  - Saturday Sunday 6:00am 12:00pm

#### Explored the following

- Utilize Colilert<sup>®</sup> and 18 hour Colilert<sup>®</sup> method for *E. coli* analyses
  - Reduce the time spent on media validation, preparation and plating
  - Better manage incubation time with readouts
- Extend the holding time of bacteria samples to > 8hrs but < 24hrs for samples not needed for compliance

# **Holding Time**

- Standard Methods On-line 9060 B: Preservation and Storage
  - Drinking Water for Compliance (Coliform) 30 hours, HPC 8 hours
  - Nonpotable water, 6 hours for transport, 2 hours for processing
  - Other water types, noncompliance < 8°C for 24 hours
- Standard Methods 9222C / 9222E: Delayed-Incubation Total Colifornin / Fecal Coliform
  - Sample is filtered in the field and placed on transport media to delay the test start for 72 hours
    - Not possible to maintain transport temperature
    - When the time between collection and analysis exceed approved holding time
    - Sampling location is remote from the laboratory
    - Stream monitoring for water quality or pollution control activities

## **Literature Review**

- Standridge and Lesar (1977), Comparison of Four-Hour and Twenty-Four-Hour Refrigerated Storage of Nonportable Water for Fecal Coliform Analysis.
  - The 14<sup>th</sup> edition of Standard Methods required samples for fecal coliform analyses to be stored at <10°C and analyzed within 8 hours of sample collection</li>
  - Authors examined whether samples could be held for 24 hours at 4°C and still produce acceptable results
  - Results for 24 of 28 samples were within the 20% variation requirement of the study; results were determined to be acceptable
- Dutka and EL-Shaarawi (1980), *Microbiological Water and Effluent Sample Preservation* 
  - Performed on 4 sample types ranging from stream water to industrial and domestic effluents
  - Storage temperature at 1.5°C, analyses performed at 2, 24, 30 and 48 hour intervals
  - Tested total and fecal coliform, and heterotrophic populations
  - Results indicated that 75% of the samples analyzed for total and fecal coliform were stable for at least 48 hours, while 50% were considered stable for HPCs

## **Literature Review**

- Standridge and Delfino (1983), Effect of Ambient Temperature Storage on Potable Water and Coliform Population Estimations
  - Study was to determine if the lengthened storage time had a negative affect on the microbial population,
  - Samples were randomly selected from routine samples collected across the State of Wisconsin
  - Samples were held at 20 ± 2°C for 24 and 48 hours and analyzed for total coliforms
  - Study indicated that samples were NOT negatively impacted
- US EPA (1985), Holding Effects on Coliform Enumeration in Drinking Water Samples
  - Study was initiated to stress the need to adhere to recommended method holding time and temperature for microbiological testing
  - The results indicated a decrease in total coliform counts, which could increase the reporting of false negatives
  - The results for heterotrophic bacteria increased by 0.5 to 2.5 orders of magnitude and interfered with coliform counts
  - Results indicate that samples should be put on ice after collection and analyzed as soon as possible

## **Literature Review**

- Pope et al., (2003), Assessment of the Effect of Holding Time and Temperature on E. coli Densities in Surface Water Samples
  - Study was sponsored by the US EPA
  - Objective was to determine if holding time and storage conditions had an effect on *E. coli* densities in surface water
  - Sample storage temperature was < 10°C</li>
  - Samples were analyzed at time 0, 8, 24, 30 and 48 hours using Colilert<sup>®</sup> and mFC agar
  - Results indicated that holding times for most surface waters samples could be extended beyond 8 hours and up to 24 hours and still produce comparable results. However, the samples should always be analyzed as soon as possible after collection.

#### Aulenbach (2010)

- Samples collected from urban streams with bacteria densities > 126 cfu/100ml
- Samples were analyzed by membrane filtration and Colilert<sup>®</sup>, holding time was extended up to 62 hours
- Results indicated that fecal and total coliform densities remained consistent up to 27 hours, while *E. coli* densities were stable up to 18 hours
- *E. coli* results differed slightly from the study performed by Pope et al., 2003.

# **Holding Time Studies**

#### Study Objectives

- Identify if samples collected for *E. coli* analysis are biologically stable for > 8 hours for various types of water samples
- Samples were collected from the following sources
  - Raw Influent High bacteria load, solids, and organic
  - Treated Effluent Low bacteria, solids and organic
  - Treated Effluent disinfected Low bacteria, solids and organic
- Analyze samples using EPA 1603 *E. coli* analysis by membrane filtration using modified mTEC agar and Colilert<sup>®</sup>
- 24, 48 hours and seven (7) day holding times at < 6°C and room temperature (25°C) compared to 8 hours (regulatory)</li>
- Identify if the use of an extended holding time would be acceptable for specific projects

## **24 Hour Holding Time**

#### Objectives

- Collect raw influent (RI) and treated effluent (TE) samples from three (3) WWTPs (Easterly, Westerly, Southerly)
- Source Information
  - Raw Influent High bacteria load, solids, and organic
  - Primary Effluent High bacteria load, moderate solids and organics
  - Treated Effluent Chlorinated Low bacteria, solids and organic
- Samples were analyzed at < 8 hours</li>
- Samples were stored at < 6°C and analyzed after 24 hours</li>
- Analysis method = Membrane Filtration

### **Results:**

- Decrease in bacteria density over-time for RI and PE sample
- Observed a *slight increase* in bacterial density with the chlorinated effluent
- The 14% increase or positive increase was similar to what was seen with the Colilert validation for chlorinated effluent





		Pearson	Average
Sample Type	P-value	Correlation	% Difference
Raw Influent	<0.05	0.89	-52%
Primary Effluent	<0.05	0.74	-48%
Treated Effluent	0.11	0.98	14%

# **NEORSD Colilert Validation Study**

- **Colilert Method Validation** 
  - Perform a validation study on the Colilert method vs Membrane Filtration (MF) using the following sample matrices.
    - Raw Influent High bacteria load, solids, and organic
    - Treated Effluent Low bacteria, solids and organic
    - Treated Effluent disinfected Low bacteria, solids and organic
  - Goal was to show that the Colilert method could provide comparable results







### **Colilert® Validation**

- Wastewaters examined
  - Treated Effluent
  - Treated Effluent Chlorinated
- Higher variability with chlorinated effluents and a slight positive effect with Colilert







## **Colilert® Validation Study Results**

#### **Colilert® vs Membrane Filtration**

- Results from the MF and Colilert<sup>®</sup> are highly correlated with the exception of chlorinated effluents
- Significant difference between MF and Colilert<sup>®</sup> (higher) for chlorinated effluents
- Consultant determined that the difference in results were acceptable and would rather error on the side of caution when determining disinfection efficiency
- Further investigate the difference with these methods in 2017
- 40 CFR Part 136 Foot Notes recommends MPN method for resolving controversial results and chlorine treated waters

## 24 - 48 Hour Holding Time

#### Objectives

- Collect raw influent (RI) and treated effluent (TE) samples from 3 WWTPs (Easterly, Westerly, Southerly)
- Source Information
  - Raw Influent High bacteria load, solids, and organic
  - Treated Effluent Moderate bacteria, solids and organic
- Samples were analyzed at < 8 hours</li>
- Samples were stored at < 6°C and analyzed after 24 hours and 48 hours</li>
- Samples were stored at room temperature (25°C) and analyzed after 24 hours and 48 hours
- Analysis method = Colilert<sup>®</sup>

### **Results: 24 - 48 Hours RI**









Sample Type	Holding Time Hrs.	P-value	Pearson Correlation	Average % Difference
Raw Influent <6°C	24	<0.05	0.57	-27%
Raw Influent <6°C	48	<0.05	0.42	-35%
Raw Influent RT	24	<0.05	0.32	-28%
Raw Influent RT	48	<0.05	0.083	-124%

#### **Results: 24 - 48 Hours TE**









Sample Type	Holding Time Hrs.	P-value	Pearson Correlation	Average % Difference
Treated Effluent <6°C	24	0.77	0.96	-7.8%
Treated Effluent <6°C	48	0.74	0.72	-12.2%
Treated Effluent RT	24	0.033	0.92	-34.5%
Treated Effluent RT	48	<0.05	0.36	-84.1%

# **Holding Time Study Results**

- Overall decrease in bacterial density with an extended holding time of > 8 hours
  - The magnitude of decrease appears to be dependent on the initial bacterial density, and perhaps organic matter present in the sample
    - Raw influents and primary effluents appear to have the greatest decrease in density over 24 and 48 hours
    - The magnitude of difference decreased and the bacterial load and organic content in the sample decreased
    - Statistical evaluation < 0.05 indicates a significant difference
  - Temperature appears to effect bacterial densities particularly when holding samples >24 hours

### **Summary**

- Greater decrease in density for raw influents and primary effluents when using MF than the Colilert<sup>®</sup> method
- Positive increase in results for chlorinated treated effluents when using MF vs Colilert<sup>®</sup>
- Treated effluent samples and
  chlorinated effluent samples appear to
  be stable for up to 24 hours and our
  data indicates the difference between
  values in not statistically significant

Sample Type	Hilding Time	P-value	Correlation	Avg %Difference
<sup>1</sup> Raw Influent <6°C	24	<0.05	0.89	-52%
Raw Influent <6°C	24	<0.05	0.57	-27%
Raw Influent RT	24	<0.05	0.32	-28%
Raw Influent <6°C	48	<0.05	0.42	-35%
Raw Influent RT	48	<0.05	0.083	-124%
<sup>1</sup> Primary Effluent <6°	24	<0.05	0.74	-48%
<sup>1,2</sup> Treated Effluent <6°C	24	0.11	0.98	14%
Treated Effluent <6°C	24	0.77	0.96	-7.8%
Treated Effluent <6°C	48	0.74	0.72	-12.2%
Treated Effluent RT	24	0.033	0.92	-34.5%
Treated Effluent RT	48	<0.05	0.36	-84.1%
<sup>1</sup> Membrane Filtration Method (MF)		<sup>2</sup> Chlorinated Effluent		

## **Future Studies**

- Explore slightly positive results for samples analyzed by membrane filtration when held for 24 hours
  - Could this be associated with the slight increase in bacteria density when using the Colilert<sup>®</sup> method.
  - Could the additional holding time allow for the recovery of stressed or injured bacteria from the chlorination process
  - Analyze BOD, TSS and COD data to see determine if the solids and organic loading correlate with reduction of bacterial density in the raw influent and primary effluent data
- Explore incremental holdtime studies (~ 4 hrs) for primary effluents.

## **Current operations**

- Extending the holding time for samples collected bacterial can be beneficial depending on the intended use of the data
- NEORSD has used extended holding times for
  - Disinfection optimization (Startup and wet weather operation)
  - Weather dependent sampling
    - CSO Modeling, Integrated planning, beach modeling
  - Large scale sampling projects for illicit discharges
  - Optimization of high rate treatment (CEHRT)

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### Combined sewer overflow (CSO)



