Rapid bacteriological quantification using defined substrate enzymatic activity in municipal wastewater

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Introduction

Project Objective

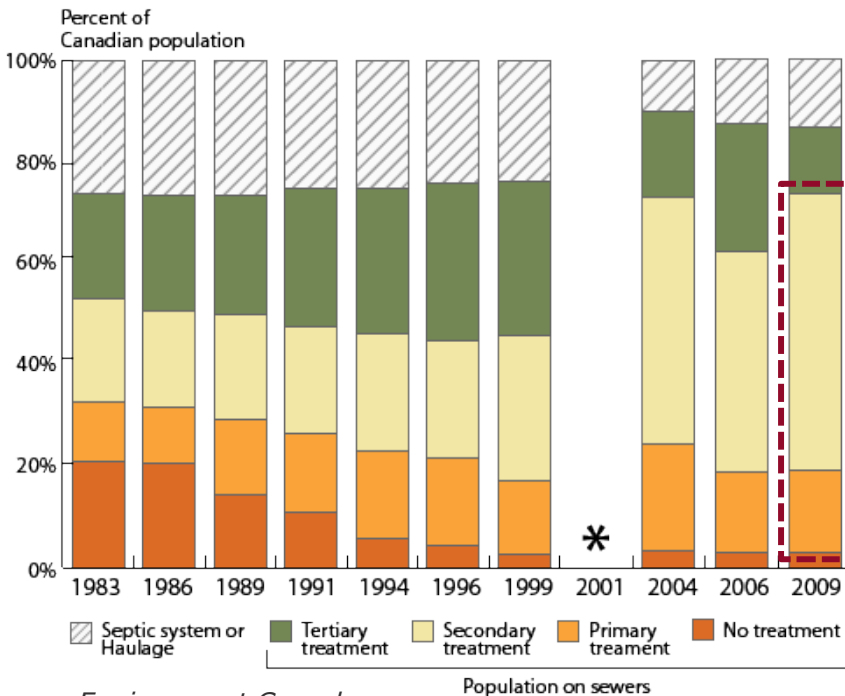- Rapid *E. coli* detection in municipal wastewater treatment systems

Field Site

- Amherstview WPCP (rated peak capacity: 16,000 m^3/day)
- South Eastern Ontario, Canada

Industry Partner & Technology

- TECTA-PDS Inc. (Formerly *Veolia Endetec*)

Wastewater Treatment Systems in Canada

Environment Canada.
2016

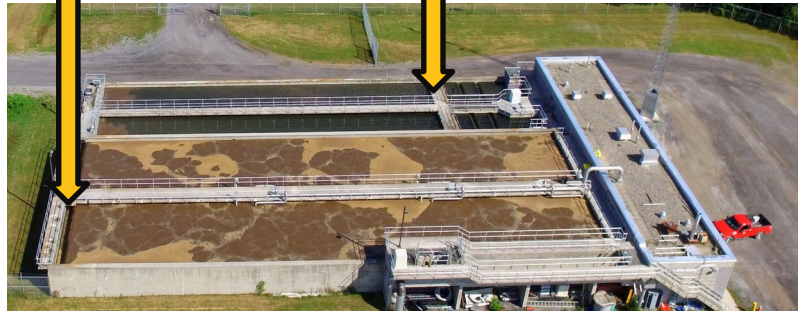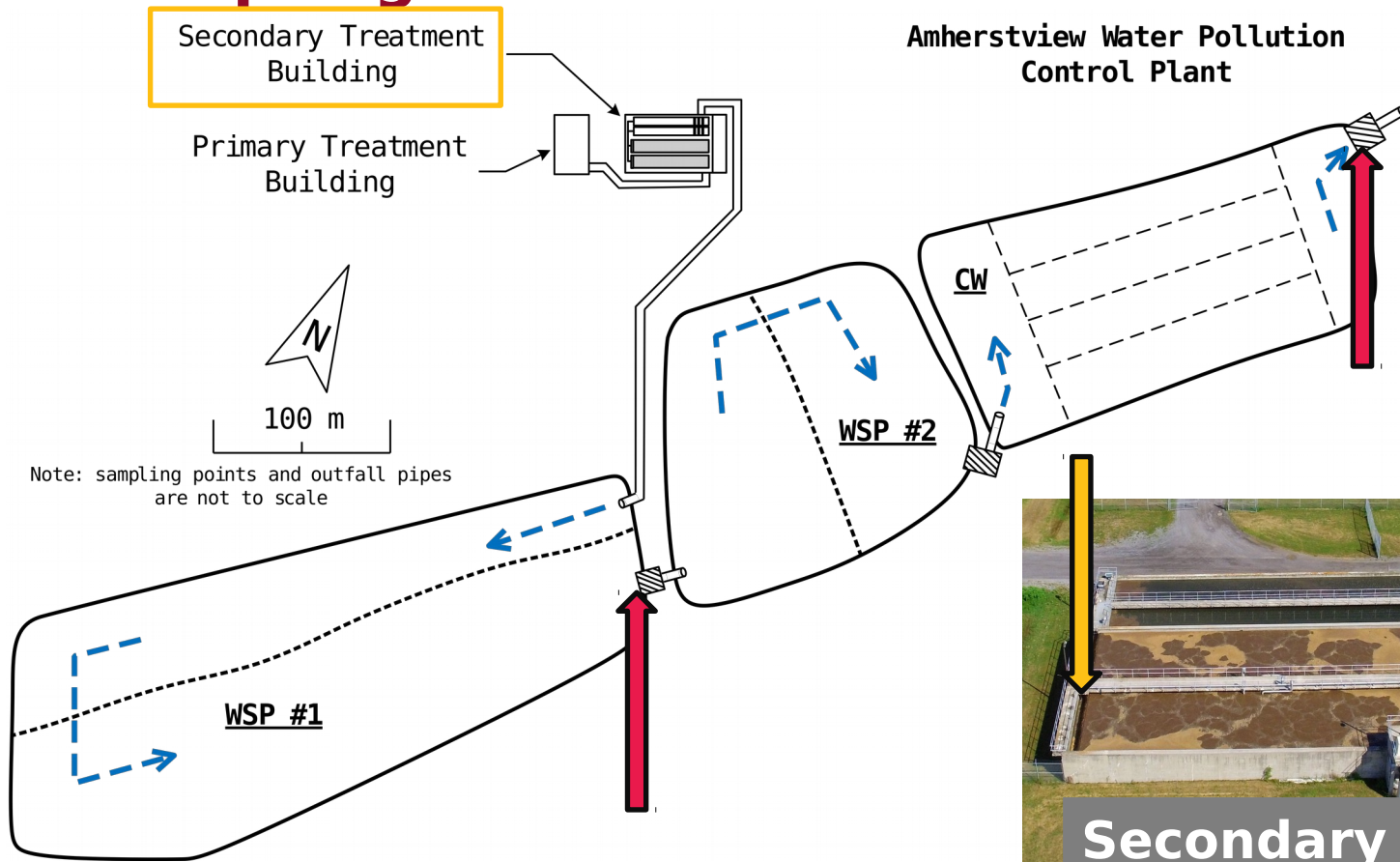## Use of lagoons

Features	> 100,000	> 25,000	> 5,000	> 1,000	< 1,000	Not Given	Total
All facilities	30	55	136	289	178	50	738
1 or more lagoon types	2	11	66	190	116	25	410
% with 1 or more lagoons	6.7	23.0	48.5	65.7	65.2	50.0	55.6
No. with Aerobic lagoons	1	5	44	117	31	12	210
No. with Anaerobic lagoons		1	9	50	46	11	117
No. with Facultative lagoons	1	6	30	52	59	5	153

Compliance Monitoring and Toxicity Testing Requirements

Facility Size	Flow Rate (m³/day)	TRC[1] (or dechlorination agent)	TSS and CBOD₅	Acute Toxicity	Chronic Toxicity
Very Small	≤ 500	Daily	Monthly[2]	n/a	n/a
Small	> 500 – 2,500	Daily	Monthly[2]	n/a	n/a
Medium	> 2,500 – 17,500	Daily	Every 2 weeks	Quarterly	Quarterly
Large	> 17,500 – 50,000	Twice per day	Weekly	Quarterly	Quarterly
Very Large	> 50,000	Three times per day	Five days per week	Monthly	Monthly

CCME Council of Ministers.
2009

3
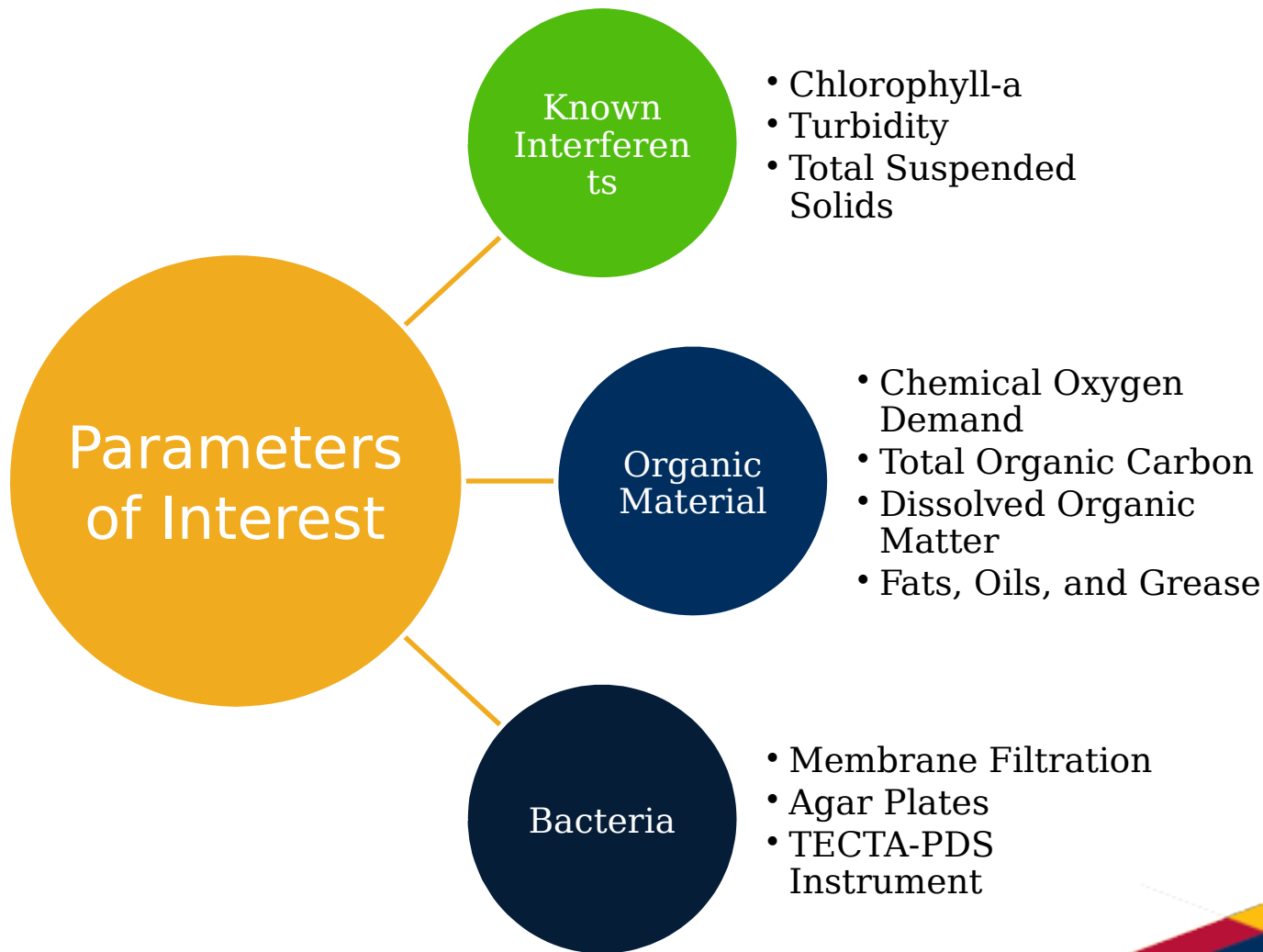
Sampling Locations

Secondary Treatment Building

Primary Treatment Building

Amherstview Water Pollution Control Plant

N

100 m

Note: sampling points and outfall pipes are not to scale

WSP #1

WSP #2

CW

Secondary Treatment

Amherstview Water Pollution Control Plant

Secondary Treatment

Waste Stabilisation Pond

Constructed Wetland

Parameters of Interest

Known Interferents
- Chlorophyll-a
- Turbidity
- Total Suspended Solids

Organic Material
- Chemical Oxygen Demand
- Total Organic Carbon
- Dissolved Organic Matter
- Fats, Oils, and Grease

Bacteria
- Membrane Filtration
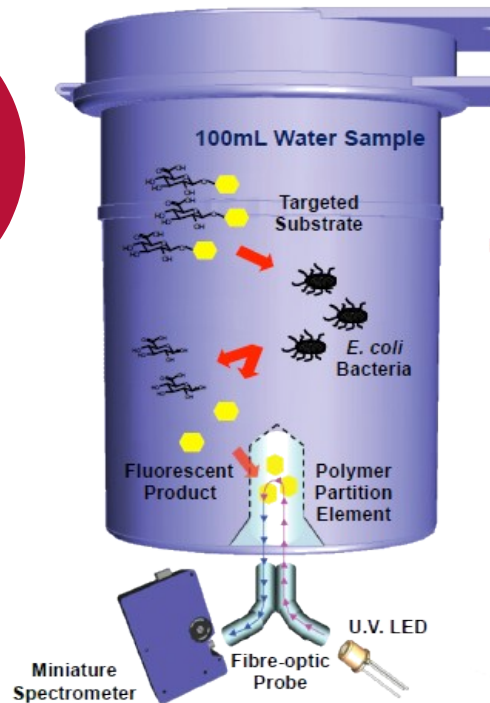- Agar Plates
- TECTA-PDS Instrument

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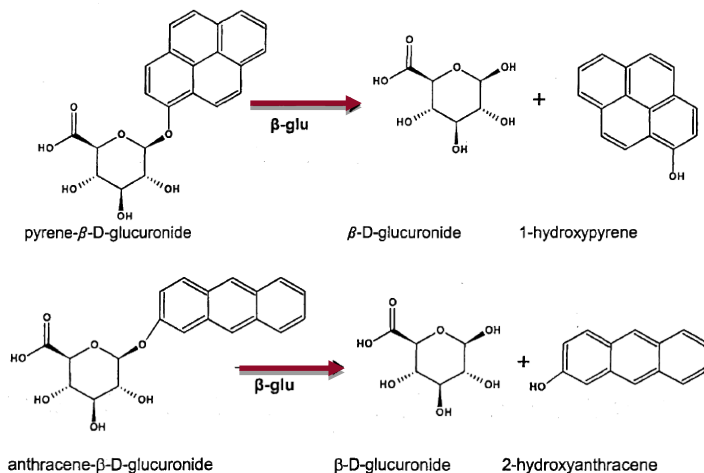- Agar Plates
- TECTA PDS Instrument

Instrument: TECTA - PDS

Enzyme produced by bacteria

+

Fluorophore bound substrate

=

Florescent signal detected

pyrene-*β*-D-glucuronide → β-D-glucuronide + 1-hydroxypyrene (β-glu)

anthracene-β-D-glucuronide → β-D-glucuronide + 2-hydroxyanthracene (β-glu)

100mL Water Sample

Targeted Substrate

E. coli Bacteria

Fluorescent Product

Polymer Partition Element

Miniature Spectrometer

Fibre-optic Probe

U.V. LED

Florescent Spectra in Wastewater

- Excitation: single wavelength
- Emission: 200-700 nm spectrum
- Recorded temporally (0-18 hr.)
- Correlative bacteria quantity to florescent "time-to-detection" (TTD)

Visualization of ideal dual-excitation, spatial and temporal spectrum


Li et. al, **2014**
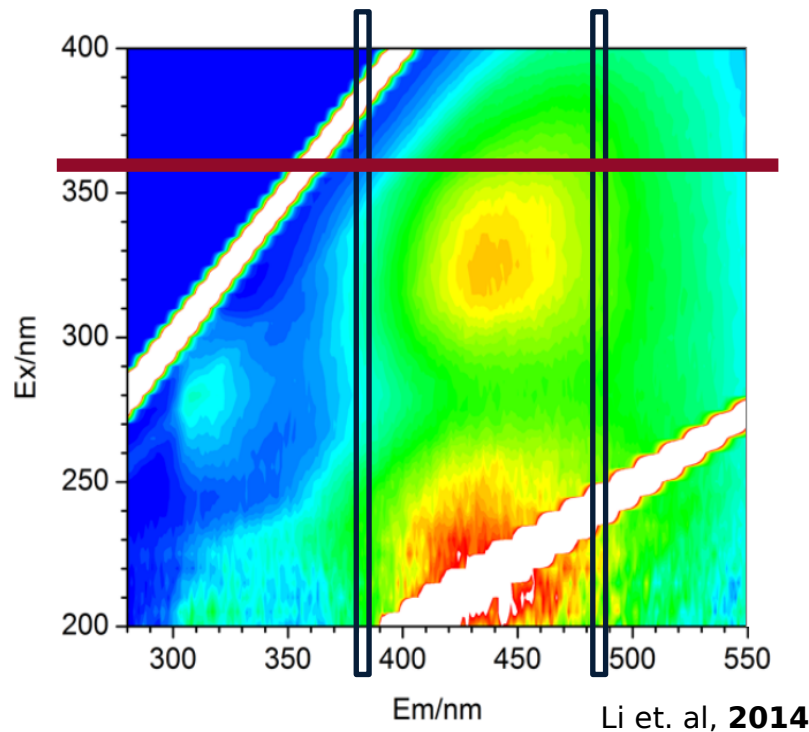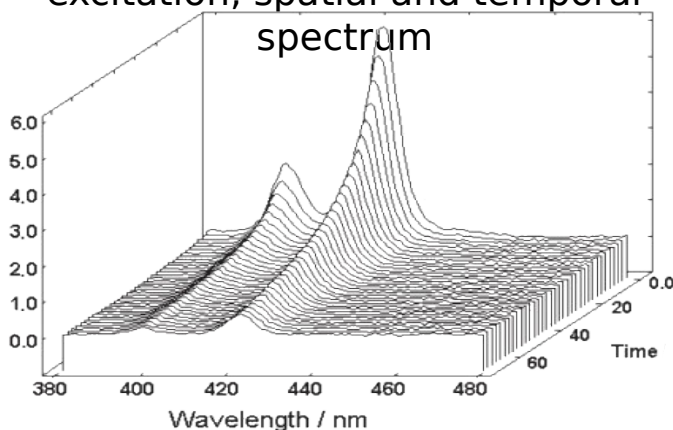
Instrument Calibration

- Correlative bacteria quantity to florescent signal
- Based on *Monod* Growth Kinetics

$$\mu = \mu_{\max} \frac{[S]}{K + [S]}$$

  - First-order rate constant particular to the exponential growth phase of cultures described
  - Not subject to substrate inhibition
  - Concentration of available substrate-binding enzyme considered proportional to the bacterial density
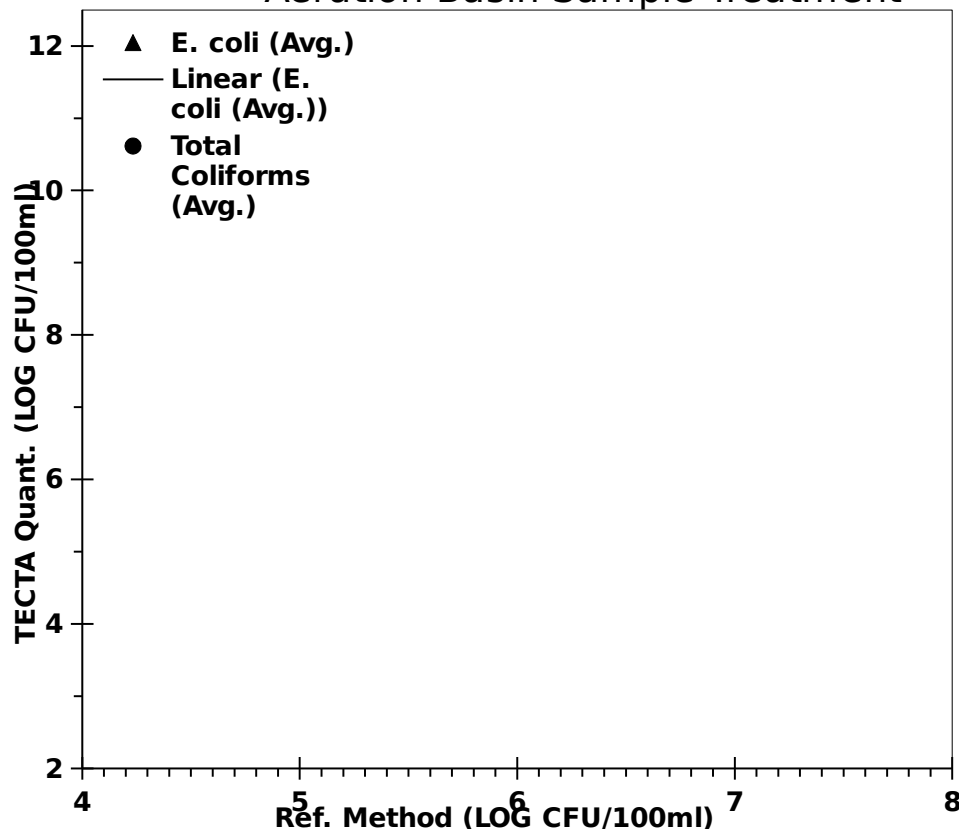- Proprietary trigger/data processing method

Brown et al. **200**

J. Monod, *Ann. Rev. Microbiol.*, 3, 371 (1949)
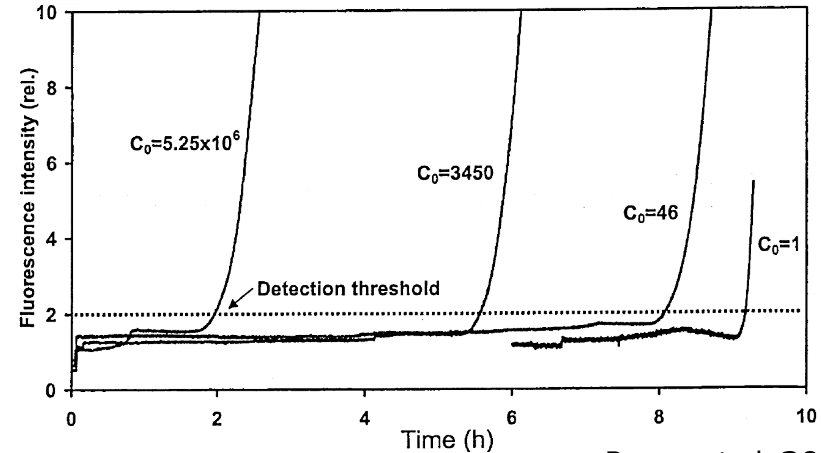
Comparison to Reference Method

Aeration Basin (Raw Sample)
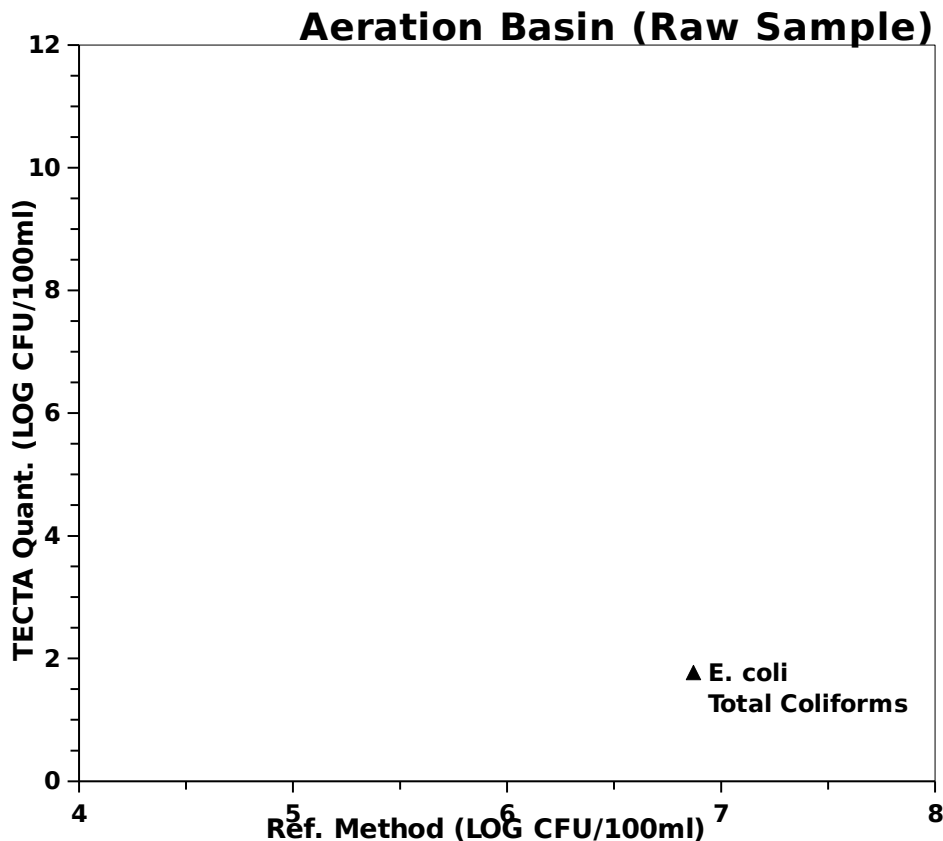
Aeration Basin	Deviation from Reference Method	
	EC	TC
	-0.1 ± 2.3	2.9 ± 0.4

- *E. coli:* high variation
- Total coliforms: low variation, high deviation from reference method
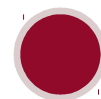- Existing method is not viable

Aeration Basin Sample Treatment

▲ E. coli (Avg.)

—— Linear (E. coli (Avg.))

● Total Coliforms (Avg.)

y-axis: TECTA Quant. (LOG CFU/100ml)

x-axis: Ref. Method (LOG CFU/100ml)

x-axis values: 4, 5, 6, 7, 8

y-axis values: 2, 4, 6, 8, 10, 12

Dilution

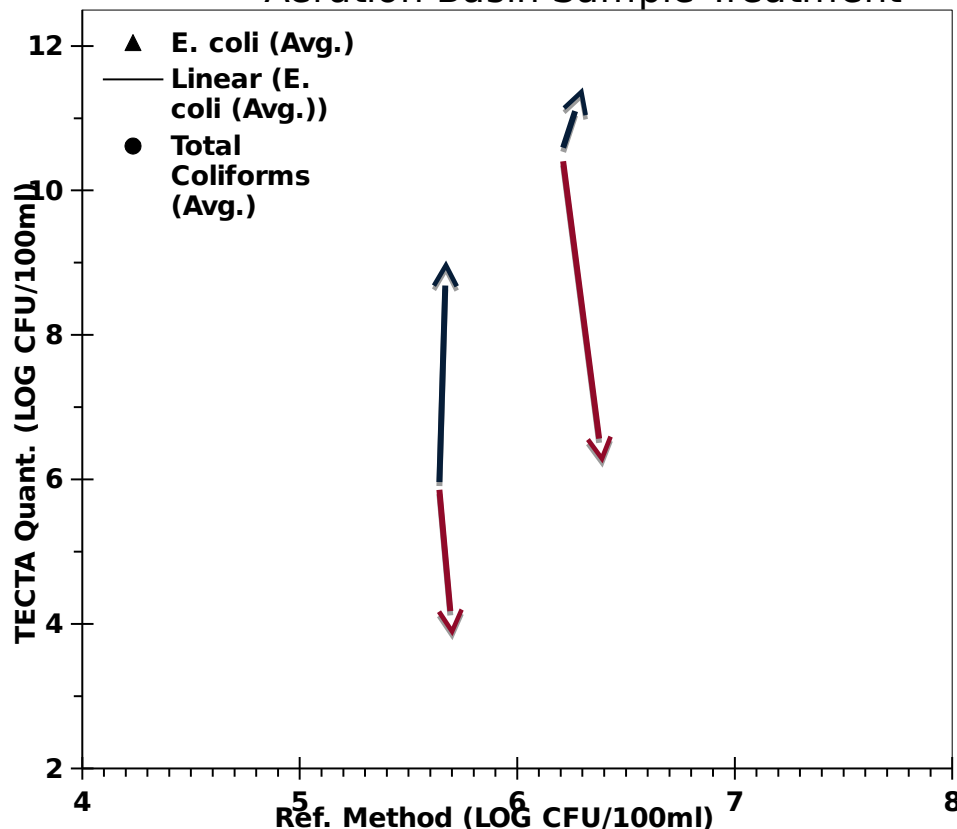Decreases all water quality parameters

Decreases amount of bacteria

Filtration

Removes suspended material

Decrease Turbidity

Chlorophyll-a
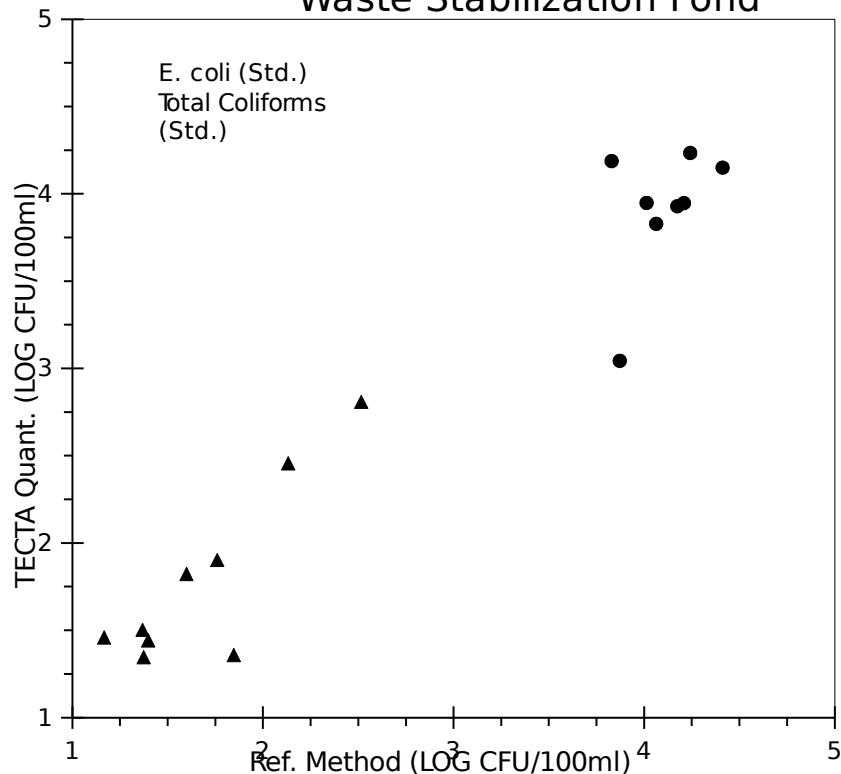
Effects of Sample Pre-treatment
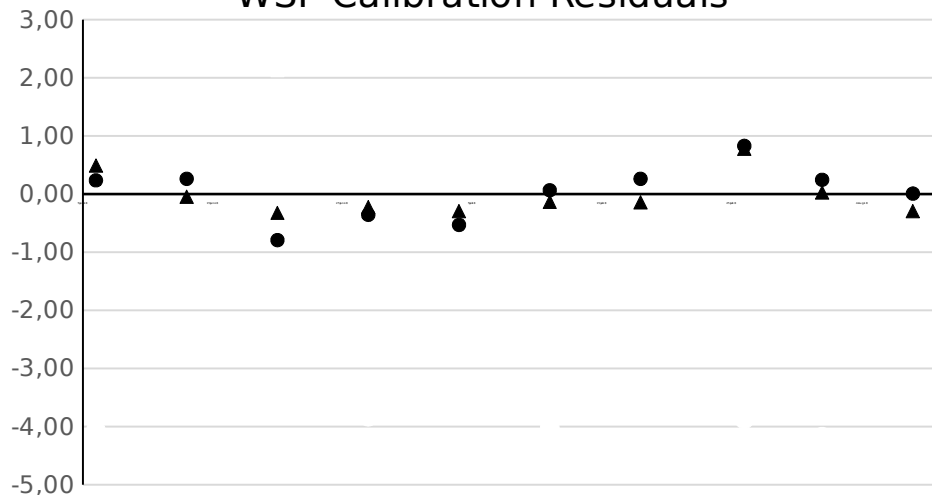
Aeration Basin Sample Treatment

Aeration Basin	Deviation from Reference Method		Avg. Detection Time (hr.)	
	EC	TC	EC	TC
Raw	0.3 ± 2.3	4.2 ± 0.4	5.06	2.95
Filtered (8 µm)	-1.8 ± 0.5	-0.1 ± 0.3	7.58	8.13
Diluted (10:1)	-3.3 ± 0.4	4.9 ± 0.4	2.57	3.34
Filter & Dilute	-1.8 ± 0.3	0.1 ± 0.4	8.55	8.88

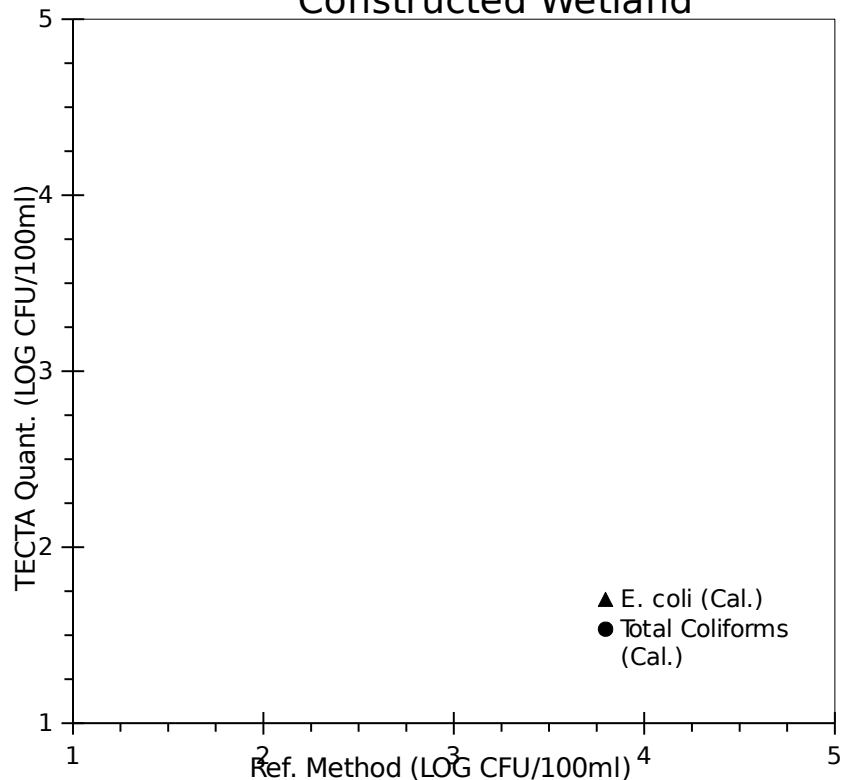# Results on Naturalized Treatment Systems

Waste Stabilization Pond

E. coli (Std.)
Total Coliforms (Std.)

TECTA Quant. (LOG CFU/100ml) vs Ref. Method (LOG CFU/100ml)

WSP Calibration Residuals

Method	Deviation from Reference Method	
	EC	TC
Standard	0.4 ± 0.4	-1.2 ± 0.8
Calibrated	0.0 ± 0.4	0.0 ± 0.8

Results on Naturalized Treatment Systems

Constructed Wetland

Calibration Residuals

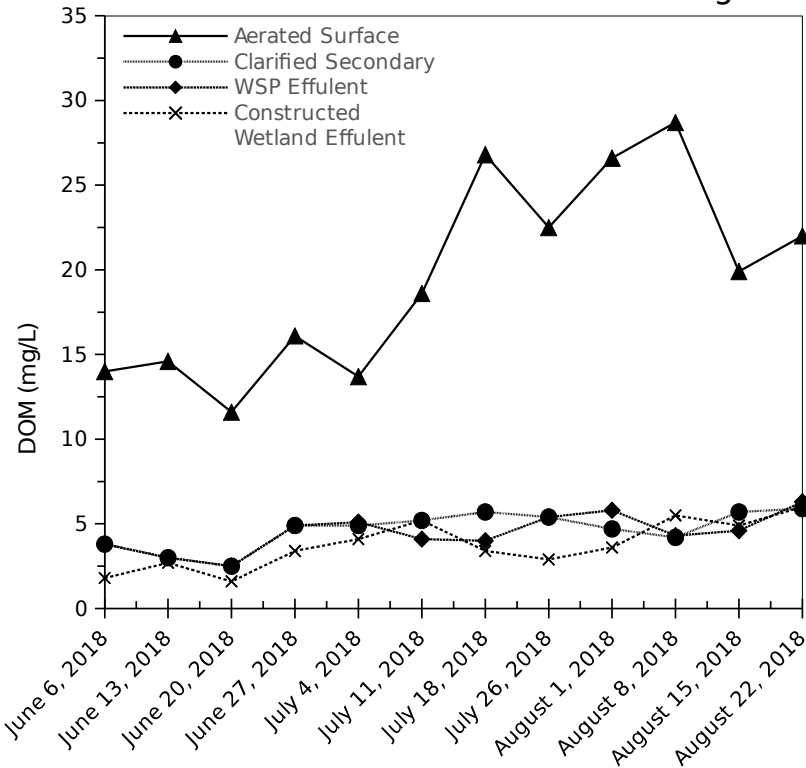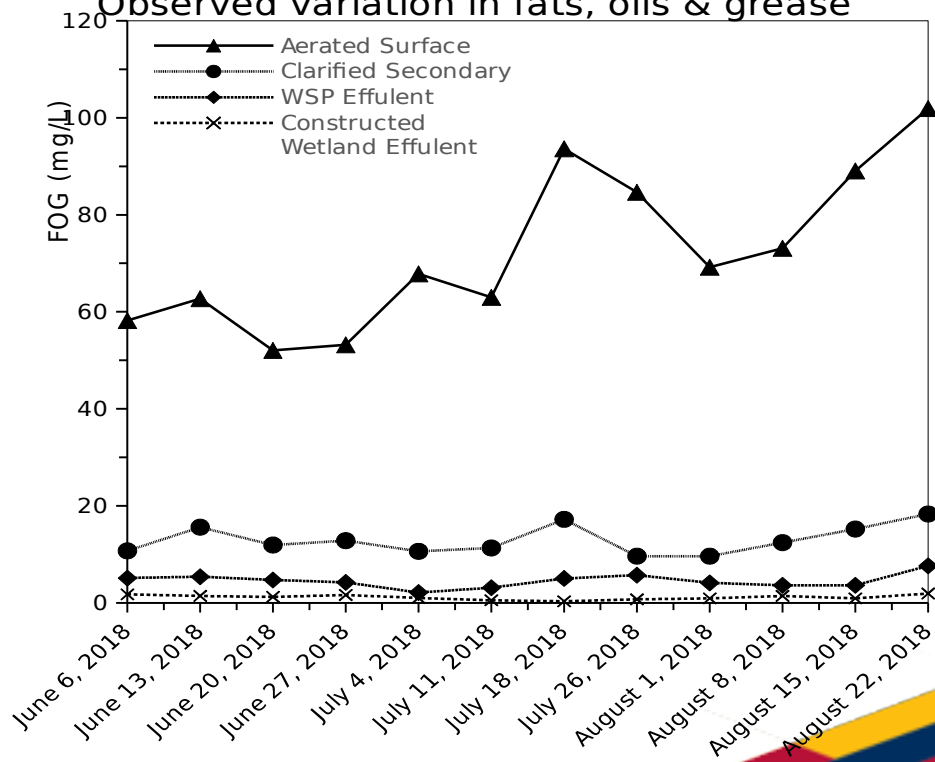| Method	Deviation from Reference Method	
	EC	TC
Standard	0.6 ± 0.5	-0.8 ± 1.3
Calibrated	0.0 ± 0.4	-0.1 ± 1.3
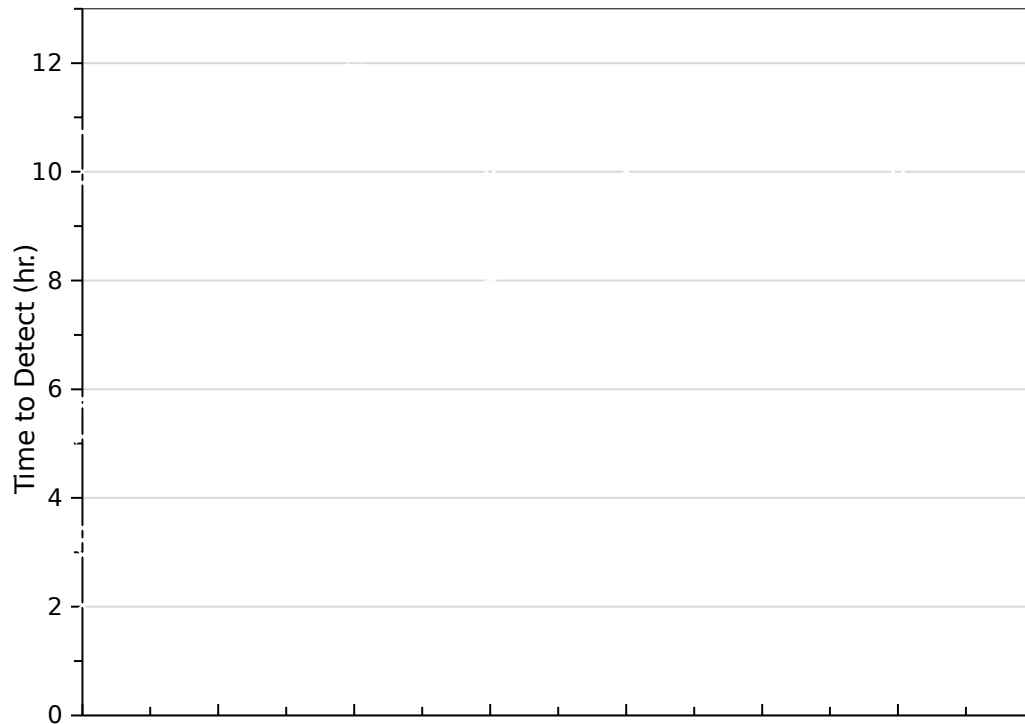
- TTD < 12h for both *E. coli* and total coliforms across all sampling points
- Potential microbial control parameter with "near-real time" qualification
- Least variance in secondary clarifier
- Less variance in total coliforms than *E. coli*

Water Characterization
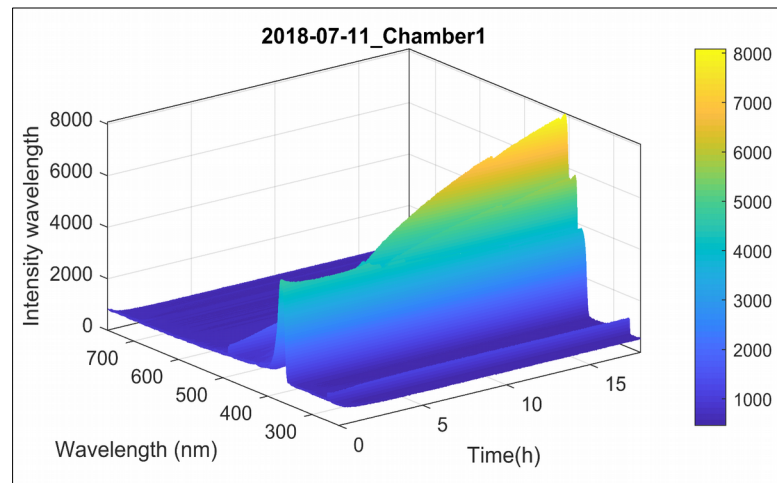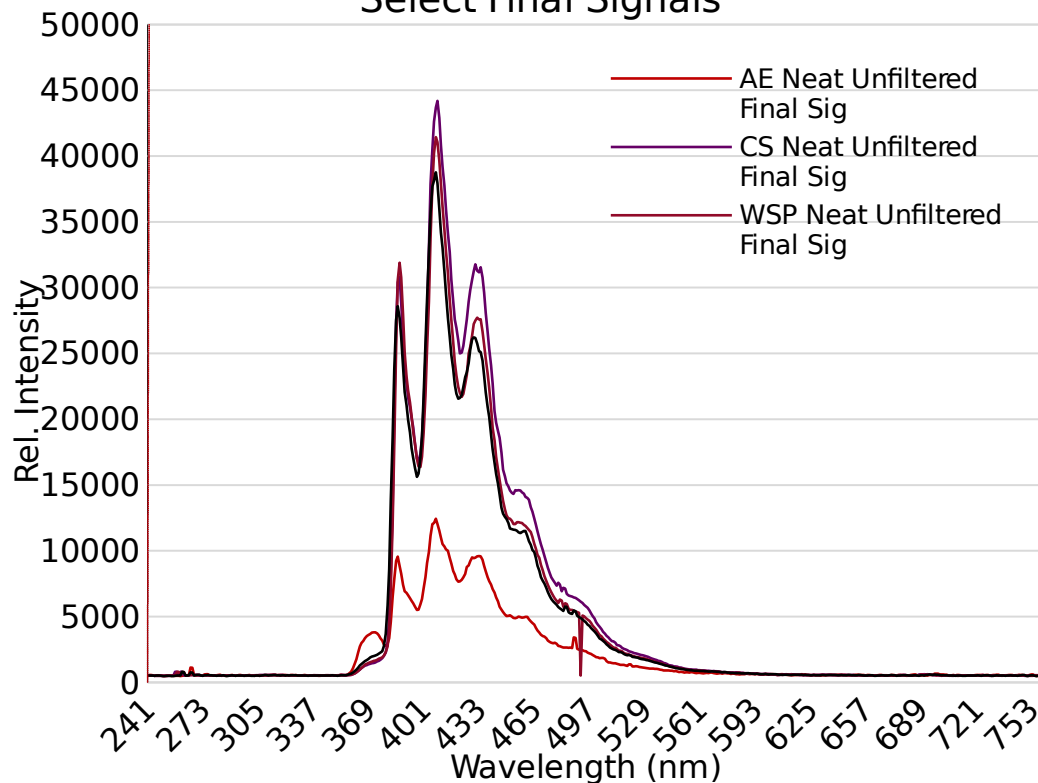

Observed variation in dissolved organic matter

Observed variation in fats, oils & grease

Evaluation of Final Spectra

Select Final Signals

- AE Neat Unfiltered Final Sig
- CS Neat Unfiltered Final Sig
- WSP Neat Unfiltered Final Sig

2018-07-11_Chamber1

Multivariate statistics show no strong-correlation, but multiple medium correlations

Confounded effects of water parameters
- Prevents statistical isolation in

Conclusions

"Near real-time" bacteria quantification	• 2-3.5 hrs in secondary treatment • 5-10 hrs in naturalized systems
Method robustness is primary dependent on water organics	• FOG levels significantly reduce signal • Insufficient signal detection above 75 mg/L
Custom Calibration	• Slope-intercept calibration improves mean but not variance in quantification • Raw vs. partially treated WW require different calibration method
Chlorophyll Interference	• Levels found in naturalized systems have little impact on florescent attenuation • Seasonal algae blooms is noticeable but insignificant in signal attenuation

Acknowledgements

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