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WATER ENVIRONMENT LABORATORY
SOLUTIONS
COVERING ANALYTICAL METHODS AND PRACTICES

A Comparison of Methods Used To Analyze a Range of Environmental Matrixes

Anne Birch and Akin Babatola

Laboratory procedures used to monitor disinfection, pollution, and contamination are continuously refined to reflect improvements in technologies and efficiencies, as well as increased or changing public health risks. Many utilities face the challenge of developing effective mechanisms to harness innovations and newer methods.

The city of Santa Cruz, Calif., located on a unique stretch of Monterey Bay, relies on tourism and ocean-associated activities to sustain its economy. The city's wastewater treatment facility (WWTF) discharges secondary-treated effluent into waters approximately 3 km (2 mi) off Monterey Bay. The facility's proximity to the bay, which is a marine sanctuary, makes it imperative to monitor discharges accurately, rapidly, and cost-effectively.

This article describes the Santa Cruz facility's experience comparing the use of the membrane filtration (MF) method with the most probable number (MPN) multiple-tube fermentation (MTF) method to analyze a variety of waters. Facility staff also compared the methods' cost-effectiveness using data gathered by the nearby City of San Jose, Calif.

Study Designed To Overcome Comparison Limitations

The Santa Cruz WWTF laboratory designed a method-development exercise to overcome major constraints encountered in many method comparisons — most prominently, matrix influences, resources, and limitations on sample size. These constraints cause many laboratories to remain entrenched in using limited monitoring technologies. This study was designed to limit the impacts of these constraints by including different environmental matrixes and analyzing samples for several months and in different seasons.

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USGS Improves Turbidity Reporting Procedures

The U.S. Geological Survey (USGS) in fall 2004 updated its procedures for turbidity data reporting to make the information more useful to users and prevent misinterpretation of data points from different types of turbidity meters. Meters that use different geometric configurations in their design can give vastly different readings for the same sample, according to a USGS press release.

"It's become fairly apparent to us that the different geometries and technologies that are used in different meters — both white light and infrared light — can and do give you sometimes drastically different numbers for the same

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Laboratory staff collected and analyzed samples from

- Neary Lagoon, a wildlife preserve adjacent to the Santa Cruz WWTP;
- WWTP secondary effluent;
- Davenport Lagoon finished water, which is used for irrigation;
- Environmental Resource Associates (Arvada, Colo.) standard samples, which typically are used for initial precision and ongoing precision; and
- storm drain samples from select locations.

All samples were analyzed simultaneously using approved MF and MTF techniques. Samples were analyzed for total and fecal coliforms and *enterococcus* for all matrixes for a 14-month period to ensure robust statistical analyses.

Sampling Methods

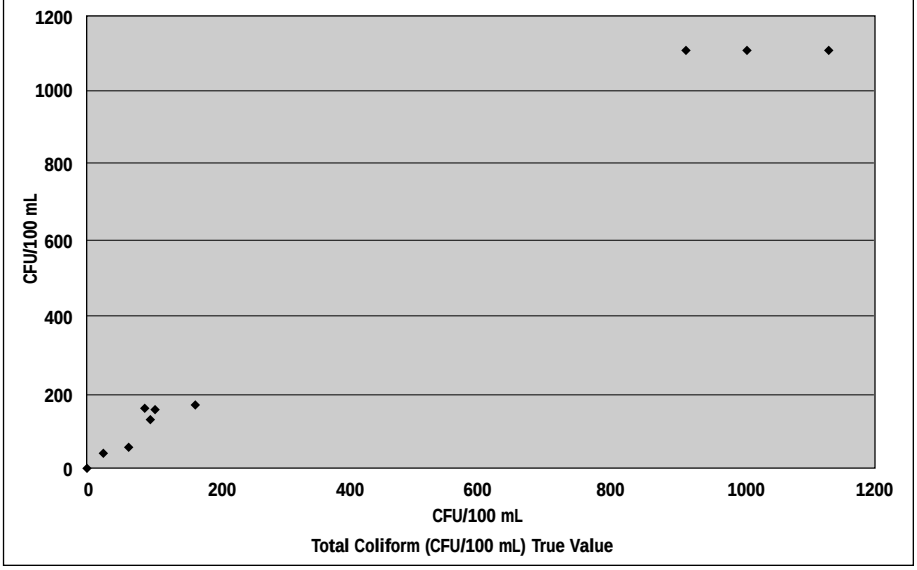
Grab samples of Neary Lagoon water were collected in Whirl-Pak bags (manufactured by Nasco of Fort Atkinson, Wis.), transported to the laboratory at the temperature range of 4˚C ± 2˚C (39.2˚F ± 3.6˚F), and analyzed on the date of collection. Grab samples of WWTF secondary effluent were collected in Whirl-Paks, typically by 11 a.m. of the sampling date, transported to the laboratory at 4˚C ± 2˚C (39.2˚F ± 3.6˚F), and analyzed on the same date. Grab samples of Davenport Lagoon water were collected in 100-mL sample jars containing 100 µl of 10% sodium thiosulfate solution (r equivalent) for dechlorination, transported to the lab at 4˚C ± 2˚C (39.2˚F ± 3.6˚F), and analyzed on the same day. Initial precision and ongoing precision samples were procured from Environmental Resource Associates and stored at 4˚C ± 2˚C (39.2˚F ± 3.6˚F) before being analyzed. Grab samples of storm drain samples were collected in Whirl-Paks and transported to the lab at 4˚C ± 2˚C (39.2˚F ± 3.6˚F).

Membrane Filtration Analyses

Millipore (Billerica. Mass.) Technology MF equipment consisting of a three-place stainless- steel filtration (manifold) unit that was autoclaved before and after each analysis was used, along with presterilized Microfil membranes and funnels with lot numbers and associated certificates of analysis, a sterile phosphate-buffered solution to dilute turbid samples, and 45-mm (1.8-in.) Petri dishes.

Following evaluation for turbidity and suspended solids, 100 mL of each sample was poured, undiluted, through presterilized funnels from Fisher Scientific International (Hampton, N.H.). High-turbidity samples (greater than 5

Figure 1.
Initial Precision/Ongoing Precision Membrane Filtration (Total Coliform)



nephelometric turbidity units) were processed by dilution through a presterilized phosphate buffer. Aliquots of 0.1 to 10 mL of these samples were processed and made up to volume using the buffer. These samples were taken from WWTF final effluent and Neary Lagoon.

Test methods were conducted in succession, after which colony-forming units were enumerated per 100 mL. For total coliform analyses, samples were collected on mEndoLES agar, covered, inverted, and incubated at 35˚C ± 0.5C (95˚F ± 0.9˚F) for 20 to 24 hours. For fecal coliform analyses, samples then were collected on mFC agar, covered, inverted, and incubated in a water bath at 44.5˚C ± 0.2˚C (112.1˚F ± 0.4˚F) for 24 hours ± 2 hours. For *enterococcus* analyses, the samples then were collected on mEnterococcus agar, covered, inverted, and incubated at 35˚C ± 0.5˚C (95˚F ± 0.9˚F) for 48 hours.

Figure 2.
Initial Precision/Ongoing Precision Membrane Filtration (Fecal Coliform)

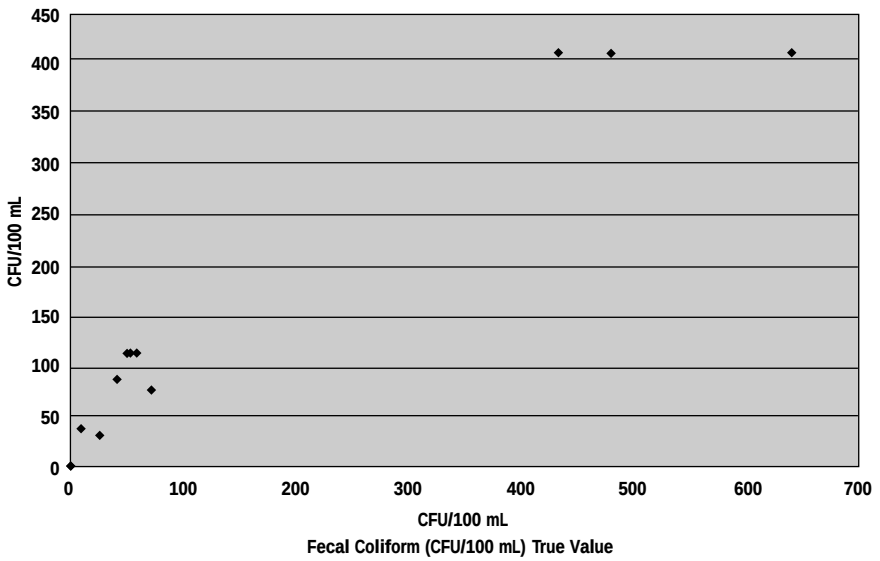
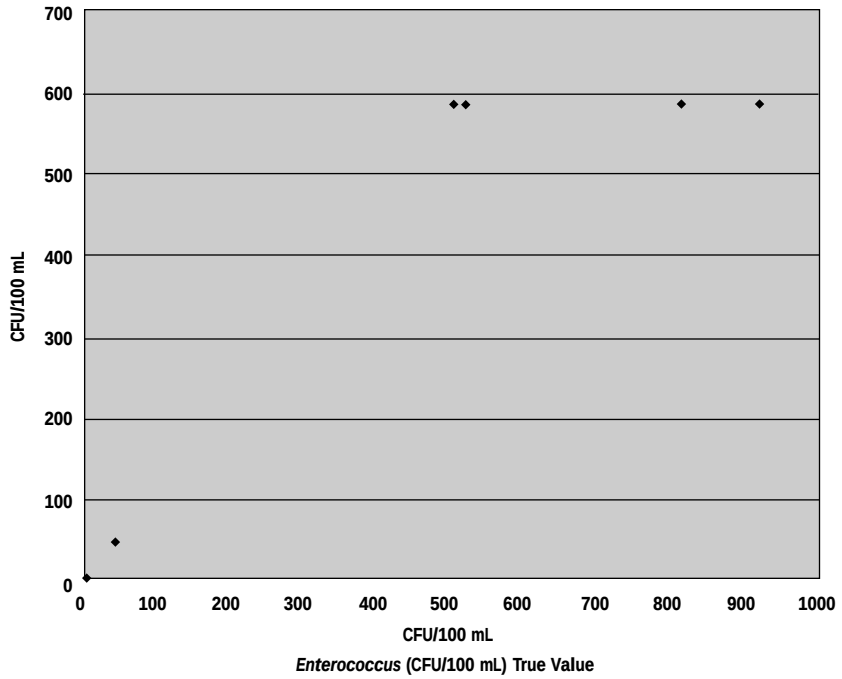


Figure 3.
Initial Precision/Ongoing Precision Membrane Filtration *(Enterococcus)*



Multiple-Tube Fermentation Analyses

MTF for total and fecal coliforms was performed in accordance with *Standard Methods for the Examination of Water and Wastewater*, 20th edition. For total coliforms, all samples underwent three serial dilutions using the lauryl tryptose broth tubes and incubated for 24 ± 2 hours at 35˚C ± 0.5˚C (95˚F ± 0.9˚F).

All tubes showing a positive reaction were transferred to confirmatory brilliant green lactose bile (BGLB) broth and incubated for 48 ± 3 hours at 35˚C ± 0.5˚C (95˚F ± 0.9˚F). Tubes showing gas production with BGLB broth were recorded, and the coliform density was estimated using the MPN table.

For fecal coliforms, all samples underwent three serial dilutions of the lauryl tryptose broth tubes for 24 ± 2 hours at 35˚C ± 0.5˚C (95˚F ± 0.9˚F). All tubes showing a positive reaction were transferred to a confirmatory EC medium broth and incubated in a water bath for 24 ± 2 hours at 44.5˚C ± 0.5˚C (112.1˚F ± 0.4˚F). Tubes showing gas production with BGLB broth were recorded, and coliform density was estimated using the MPN table.

Results

The table (p. 4) and figures 1–3 (p. 2-3) illustrate laboratory proficiencies in MF. Statistical analysis of the data showed good correlation between the two procedures for all three bacterial indicators and for all matrixes. R^2 values ranged from 0.62 to 0.90 ($p < 0.0001$). P values of < 0.05 indicated a significant correlation, while r^2 values in excess of 0.6 implied that one method was a good predictor of the other method.

Comparison of Membrane Filtration Results and Standard Levels, 2003–2004

		Total coliforms (CFU/100 mL)		Fecal coliforms (CFU/100 mL)		*Enterococcus* (CFU/100 mL)	
Date	**ERA Sample Name**	**Membrane filtration**	**True value**	**Membrane filtration**	**True value**	**Membrane filtration**	**True value**
5/18/2003	WP-99	28	44	10	38		
	ERA QC-A	98	131	42	87		
	ERA QC-B	<1.0	<1.0	<1.0	<1.0		
7/22/2003	ERA QC-A	104	160	54	113		
	ERA QC-B	<1.0	<1.0	<1.0	<1.0		
10/13/2003	ERA QC-A	92	160	60	113		
	ERA QC-B	<1.0	<1.0	<1.0	<1.0		
11/24/2003	ERA QC-A	90	160	52	113		
	ERA QC-B	<1.0	<1.0	<1.0	<1.0		
1/11/2004	ERA QC-A	1130	1110	640	411	525	587
	ERA QC-B	<2	<1.0	<2	<1.0	<1.0	<1.0
2/16/2004	ERA QC-A	1005	1110	480	411	510	587
	ERA QC-B	<2	<1.0	<2	<1.0	<1.0	<1.0
3/29/2004	ERA QC-A	914	1110	433	411	820	587
	ERA QC-B	<2	<1.0	<2	<1.0	<1.0	<1.0
5/3/2004	ERA QC-A	167	171	73	77	925	587
	ERA QC-B	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
	WP-111	65	59		27	32	49

CFU = colony forming unit.
WP = blind samples purchased to tests a lab's proficiency.
QC = blind, quality control sample purchased and analyzed to assess quality control measures.

Although it has been established that the MF is a more rapid, cost-effective, and precise method for analyzing total coliforms in chlorinated tertiary effluents, its application to other environmental matrixes was limited by sample size and data quality. However, this project managed to provide data comparing MF and MPN methods for fecal and total coliforms and *enterococcus* in several environmental matrixes in and around Santa Cruz.

This study confirms the Santa Cruz laboratory's ability to use the MF method across a wide spectrum of environmental matrixes. The turnaround time for the MF method is much faster than for the MTF method, which is more labor-intensive and yields only a statistically probable value with a built-in positive bias. Thus, the MF method yields better precision and improved turnaround time. Also, reduction in media used translates to projected savings of more than $5000 per year. Method choices for microbial monitoring across these matrixes therefore will be predicated on project and data quality objectives and cost constraints.

Anne Birch *is a chemist and* ***Akin Babatola*** *is the laboratory–pretreatment manager at the City of Santa Cruz (Calif.) Wastewater Treatment Facility Laboratory.*

USGS Improves Turbidity Reporting Procedures *continued from page 1*

environmental sample," said G. Douglas Glysson, water quality and sedimentation specialist at USGS. Even though all meters are calibrated to the chemical formazin or a formazin-based standard, the measurements obtained with different types and models of meters can vary widely for the same environmental samples, he explained.

USGS defines *turbidity* as "an expression of the optical properties of a liquid that causes light rays to be scattered and absorbed rather than transmitted in straight lines through a sample," Glysson said. According to the definition, he said, "turbidity of water is caused by the presence of suspended and dissolved matter such as clay, silt, finely divided organic