2011

Evaluation of Select Sensors for Real-Time Monitoring of *Escherichia coli* in Water Distribution Systems

Syreeta L. Miles
University of Arizona, Tucson, Arizona

Ryan G. Sinclair
University of Arizona, Tucson, Arizona

Mark R. Riley
University of Nebraska-Lincoln, mriley3@unl.edu

Ian L. Pepper
University of Arizona, Tucson, Arizona

Applied and Environmental Microbiology, Apr. 2011, p. 2813–2816 Vol. 77, No. 8
0099-2240/11/$12.00 doi:10.1128/AEM.02618-10

Evaluation of Select Sensors for Real-Time Monitoring of *Escherichia coli* in Water Distribution Systems▽

Syreeta L. Miles,[1]* Ryan G. Sinclair,[1]† Mark R. Riley,[2] and Ian L. Pepper[1]

Soil, Water, and Environmental Science Department[1] *and Agricultural and Biosystems Engineering Department,*[2] *University of Arizona, Tucson, Arizona*

Received 8 November 2010/Accepted 16 February 2011

This study evaluated real-time sensing of *Escherichia coli* as a microbial contaminant in water distribution systems. Most sensors responded to increased *E. coli* concentrations, showing that select sensors can detect microbial water quality changes and be utilized as part of a contaminant warning system.

Monitoring water distribution systems (DSs) for a microbial intrusion has been a challenge for water utilities. Many contaminants that can cause degradation in the water quality in DSs are not monitored, and contamination events are difficult to detect due to the low frequency of required samplings (6). As a consequence, DSs are relatively unprotected and vulnerable to intentional, natural, or accidental contamination from microbial agents (5). Therefore, there is a need for real-time monitoring to recognize water quality disturbances. To date there are a limited number of studies that have evaluated the use of commercial sensors for real-time monitoring of DSs, and even fewer that demonstrate how sensors respond to microbial contaminants. The objective of this study was to evaluate the potential of multiple water quality sensors for real-time monitoring of *E. coli* as a surrogate for microbial contamination.

At the University of Arizona Real Time Monitoring Laboratory, water is delivered by the City of Tucson Water public utility. Deionized (DI) water as a control or prefiltered tap water (1-μm pore size) was used during experiments, and a baseline output from the sensors was established. Sensors were arranged in parallel and challenged with two or more replicates of *E. coli* (ATCC 15597) at a final concentration of 10^3, 10^4, 10^5, or 10^6 CFU/ml. The experiments used late-log-phase *E. coli* suspended in either tryptic soy broth (TSB) or phosphate-buffered saline (Sigma catalog no. P3813), termed "washed" (centrifuged three times at 4,000 rpm for 25 min). Water samples were obtained throughout the injection to confirm cultural analysis (by dilution, plating, and incubating at 37°C for 24 h on tryptic soy agar [BD catalog no. 236920]) or total cell analysis (by acridine orange direct count [AODC] [4]). A separate set of experiments neutralized the chlorine residual to allow for determination of the *E. coli* concentrations.

Sensors evaluated included the Hach GuardianBlue event detection system, the BioSentry technology, the S::CAN Spectrolyser technology, and the GE 5310 online total organic carbon (TOC) unit. The BioSentry was the only sensor with the potential to detect microbial contaminants (categorizing particulates as rods, spores, protozoa, and unknown) and therefore was also evaluated to determine if it could differentiate turbidity-causing particulates from *E. coli*. *E. coli* at 10^4 and 10^5 CFU/ml with the addition of 0.3 nephelometric turbidity units (NTU) (Ricca Chemical catalog no. 8830-32) was evaluated for any increase in BioSentry response above the actual *E. coli* concentration due to the turbidity added to DI water.

Most parameters analyzed in this study exhibited an increase in response to an increase in *E. coli* in TSB or washed regardless of whether DI or tap water was utilized in the DS (Tables 1 and 2). In contrast, chlorine levels decreased due to the increased organic matter. A decrease in chlorine was observed only at concentrations above 10^3 CFU/ml with *E. coli* in TSB and 10^5 CFU/ml with washed *E. coli*. A decrease in chlorine also occurred in other studies (1, 2, 3, and 7).

The numerical value of the BioSentry output for the rod category (organisms/ml) correlated well with the numerical values from culture (CFU/ml) and AODC (cells/ml) analyses, with R^2 values above 0.95. However, the R^2 value for washed *E. coli* decreased to 0.78 and 0.61 for the culture and AODC methods, respectively, in tap water. Similar high correlations were also obtained in a study performed by the EPA (7). Comparing cultural and AODC analyses at 10^3 CFU/ml showed a slight difference with BioSentry for washed *E. coli* and *E. coli* in TSB in DI water and washed *E. coli* in tap water (Fig. 1 and 2). A slight difference was also seen with both washed *E. coli* and *E. coli* in TSB in tap water at 10^6 CFU/ml (Fig. 1 and 2). Turbidity experiments demonstrated that the BioSentry output increased above the actual *E. coli* concentra-

* Corresponding author. Mailing address: University of Arizona, 2601 E. Airport Dr., Tucson, AZ 85756. Phone: (520) 626-3325. Fax: (520) 573-0852. E-mail: smiles@email.arizona.edu.

† Department of Environmental and Occupational Health, Loma Linda University, Loma Linda, CA 92350.

▽ Published ahead of print on 25 February 2011.

TABLE 1. Sensor values for *E. coli* in tap water[a]

Cell type	Target concn (CFU/ml)	BioSentry concn (org/ml)	GE TOC (mg/liter)	Hach				S::CAN		
				Turbidity (NTU)	Conductivity (μS/cm)	TOC (mg/liter)	Chlorine (mg/liter)	Turbidity (FTU) eq	DOC (mg/liter) eq	TOC (mg/liter) eq
Washed	1.0E + 03	2.4+E04 ± 8.1+E03 (1.2)	0.18 ± 0.01 (0.02)	0.13 ± 0.02 (−0.23)	615.24 ± 11.4 (0.34)	IN	0.80 ± 0.00 (−0.06)	0.38 ± 0.05 (0.20)	0.35 ± 0.0 (0.41)	4.5 ± 0.01 (7.0)
	1.0E + 04	4.8+E04 ± 1.8+E04 (3.4)	0.13 ± 0.00 (−0.09)	0.42 ± 0.26 (2.28)	712.72 ± 5.4 (0.14)	IN	0.83 ± 0.00 (−0.03)	0.39 ± 0.01 (0.41)	0.34 ± 0.01 (0.52)	4.6 ± 0.03 (5.3)
	1.0E + 05	1.5+E05 ± 1.4+E04 (14.34)	0.18 ± 0.00 (0.20)	0.21 ± 0.02 (0.62)	712.54 ± 0.62 (0.14)	IN	0.81 ± 0.00 (−0.05)	0.41 ± 0.01 (0.47)	0.39 ± 0.0 (0.60)	4.6 ± 0.0 (2.6)
	1.0E + 06	1.9+E05 ± 2.6+E04 (20.66)	0.43 ± 0.01 (1.21)	0.38 ± 0.06 (−0.36)	702.17 ± 2.5 (0.15)	IN	0.65 ± 0.02 (0.01)	0.81 ± 0.01 (3.7)	0.77 ± 0.0 (2.0)	NM
TSB	1.0E + 03	1.5+E04 ± 1.5+E04 (0.53)	0.19 ± 0.05 (−0.04)	0.07 ± 0.05 (0.05)	636.01 ± 136.3 (0.14)	1.8 ± 0.68 (−0.03)	0.83 ± 0.00 (0.00)	0.38 ± 0.05 (0.09)	0.37 ± 0.03 (0.27)	4.5 ± 0.04 (7.0)
	1.0E + 04	1.4+E04 ± 4.8+E03 (1.1)	0.27 ± 0.00 (0.26)	0.10 ± 0.01 (−0.04)	845.56 ± 1.6 (0.34)	3.6 ± 0.05 (0.03)	0.76 ± 1.37 (−0.09)	0.39 ± 0.02 (0.09)	0.36 ± 0.0 (−12.9)	4.5 ± 0.01 (6.5)
	1.0E + 05	3.0+E04 ± 1.0+E03 (4.7)	0.68 ± 0.02 (1.63)	0.09 ± 0.00 (−0.06)	871.83 ± 13.7 (0.21)	4.5 ± 0.43 (0.10)	0.56 ± 0.02 (−0.32)	0.41 ± 0.01 (0.24)	0.39 ± 0.0 (−11.9)	4.6 ± 0.01 (6.4)
	1.0E + 06	1.8+E05 ± 1.2+E04 (20.96)	3.9 ± 0.05 (11.81)	0.14 ± 0.02 (0.07)	777.02 ± 46.94 (0.07)	7.0 ± 0.39 (0.39)	0.56 ± 0.01 (−0.32)	0.81 ± 0.01 (1.8)	0.63 ± 0.01 (−18.7)	4.8 ± 0.0 (6.8)

[a] Values are averages ± standard deviations; values in parentheses are dimensionless normalized values [calculated as $\Delta I = (I - I_0)/I_0$, where I is the average signal value at maximal response and I_0 is the baseline signal value at the beginning of experiment]. Positive and negative values indicate the magnitude of the increase or decrease in the response of the sensor from background level. IN, invalid measurement; NM, not measured due to saturation.

TABLE 2. Sensor values for *E. coli* in DI water[a]

Cell type	Target concn (CFU/ml)	BioSentry concn (org/ml)	GE TOC (mg/liter)	Hach		S::CAN		
				Turbidity (NTU)	Conductivity (μS/cm)	Turbidity (FTU) eq	DOC (mg/liter) eq	TOC (mg/liter) eq
Washed	1.00E + 03	4.7+E03 ± 5.1+E03 (3.2)	0.03 ± 0.00 (−0.10)	0.24 ± 0.00 (0.00)	1.18 ± 0.03 (3.64)	0.19 ± 0.02 (0.05)	0.20 ± 0.01 (−0.03)	0.26 ± 0.00 (−0.01)
	1.00E + 04	3.5+E04 ± 4.0+E04 (22.27)	0.04 ± 0.01 (0.04)	0.14 ± 0.00 (0.00)	0.58 ± 0.01 (3.07)	0.18 ± 0.03 (0.18)	0.20 ± 0.01 (0.02)	0.26 ± 0.00 (0.04)
	1.00E + 05	6.3+E04 ± 1.1+E04 (27.48)	0.07 ± 0.02 (0.26)	0.14 ± 0.12 (0.53)	7.3 ± 3.5 (17.41)	0.26 ± 0.09 (0.06)	0.23 ± 0.03 (0.06)	0.29 ± 0.03 (0.13)
	1.00E + 06	2.9+E05 ± 7.3+E04 (176.34)	0.56 ± 0.36 (5.53)	0.24 ± 0.01 (6.77)	24.7 ± 14.1 (1.18)	1.4 ± 0.74 (3.9)	0.50 ± 0.14 (1.3)	0.98 ± 0.60 (1.3)
TSB	1.00E + 03	3.3+E03 ± 1.1+E03 (0.86)	0.10 ± 0.04 (−0.10)	0.39 ± 0.20 (0.01)	2.2 ± 1.21 (1.84)	0.20 ± 0.08 (−0.07)	0.20 ± 0.01 (0.03)	0.26 ± 0.03 (0.04)
	1.00E + 04	9.4+E03 ± 6.3+E02 (2.7)	0.13 ± 0.00 (2.10)	0.23 ± 0.01 (0.02)	1.4 ± 0.04 (3.04)	0.26 ± 0.00 (0.04)	0.20 ± 0.00 (0.06)	0.25 ± 0.00 (0.05)
	1.00E + 05	1.2+E05 ± 4.7+E04 (10.7)	0.98 ± 0.03 (15.6)	0.12 ± 0.09 (0.86)	1.7 ± 0.87 (1.65)	0.31 ± 0.01 (0.33)	0.25 ± 0.00 (0.16)	0.33 ± 0.01 (0.31)
	1.00E + 06	3.5+E05 ± 1.01+E05 (98.90)	9.5 ± 0.37 (68.8)	0.21 ± 0.01 (3.48)	12.9 ± 1.07 (22.10)	0.94 ± 0.03 (3.2)	0.76 ± 0.01 (2.8)	0.98 ± 0.04 (2.0)

[a] Values are averages ± standard deviations; values in parentheses are dimensionless normalized values [calculated as $\Delta I = (I - I_0)/I_0$, where I is the average signal value at maximal response and I_0 is the baseline signal value at the beginning of experiment]. Positive and negative values indicate the magnitude of the increase or decrease in the response of the sensor from background level.

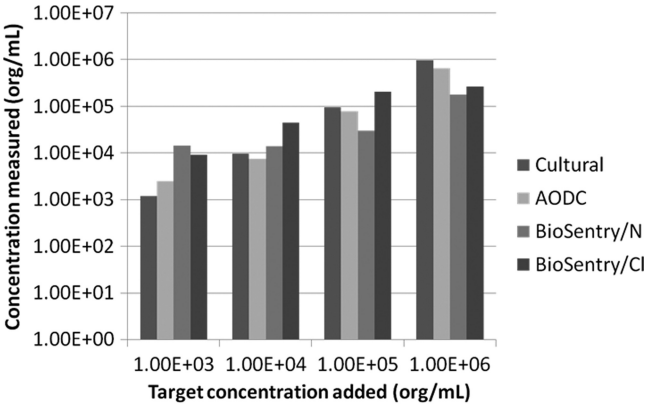

FIG. 1. Tap water with an intrusion of *E. coli* in TSB at the Real Time Monitoring Laboratory. org, organisms.

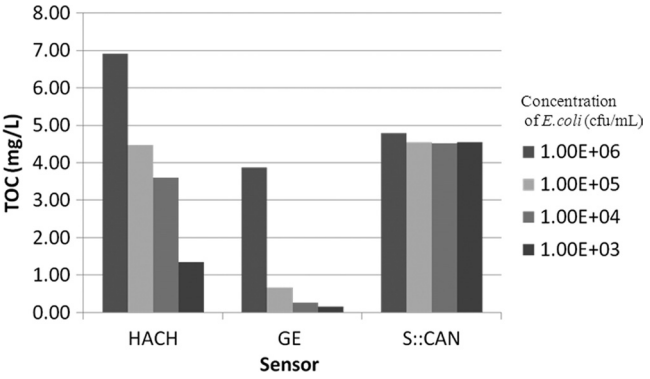

FIG. 3. Total organic carbon measured in tap water with an intrusion of *E. coli* in TSB at the Real Time Monitoring Laboratory.

tion by 38% from 2.1×10^4 org/ml to 2.9×10^4 org/ml and by 50% from 1.4×10^5 org/ml to 1.5×10^5 org/ml with the addition of a turbidity suspension at 0.3 NTU.

A comparison of TOC measurements shows that in DI water, most TOC measurements increased as *E. coli* concentrations in TSB increased for the Hach and GE units. However, the S::CAN responded only to *E. coli* in TSB above 10^6 CFU/ml and washed *E. coli* above 10^6 CFU/ml in DI. In tap water, there was an increase in TOC measurements with an increase in concentrations for *E. coli* in TSB for the Hach and GE units, but there was little response throughout the range under the same conditions for the S::CAN sensor (Fig. 3). Again there was a response to washed *E. coli* only above 10^6 CFU/ml for the GE unit, and a minimal response was seen for the S::CAN sensor in tap water (Fig. 4).

This study determined the sensitivity and threshold levels of four commercial sensors during an *E. coli* intrusion into a DS. It should be noted that detection is limited by saturation from sensor light-scattering measurements and by background noise at the upper and lower limits, respectively. The BioSentry was fairly accurate; however, the sensor could not distinguish between particulates and *E. coli* if concentrations were relatively high, and therefore it is critical to investigate the cause of an increase to negate the possibility of false positives. The response of the TOC sensors to intrusion events was variable, particularly when washed *E. coli* was introduced, and TOC sensors were also more sensitive in the detection of the media associated with *E. coli* than the microorganisms themselves. Similar conclusions have also been made in other studies for TOC sensors (1, 7).

An overall evaluation of all response data from different experiments validates the necessity for multiple sensors with different modes of action to ensure detection of different contaminants. Although from a drinking water perspective, the sensitivity of the sensors for the detection of *E. coli* could be viewed as poor, these sensors did improve the monitoring frequency of the microbial water quality considerably and could be implemented into a supervisory control and data acquisition (SCADA) system to introduce algorithms that would increase the sensitivity of detection.

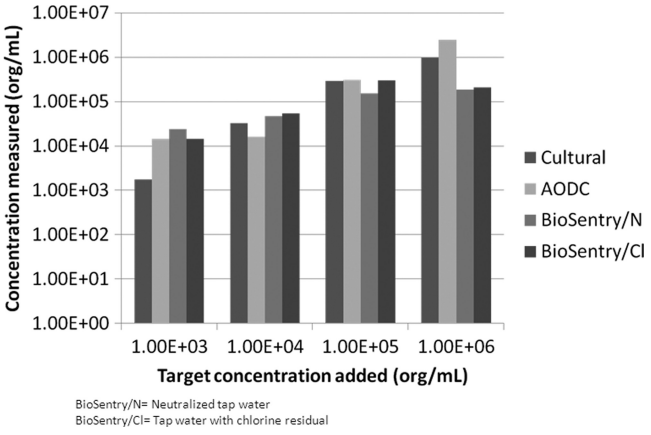

FIG. 2. Tap water with an intrusion of washed *E. coli* at the Real Time Monitoring Laboratory. org, organisms.

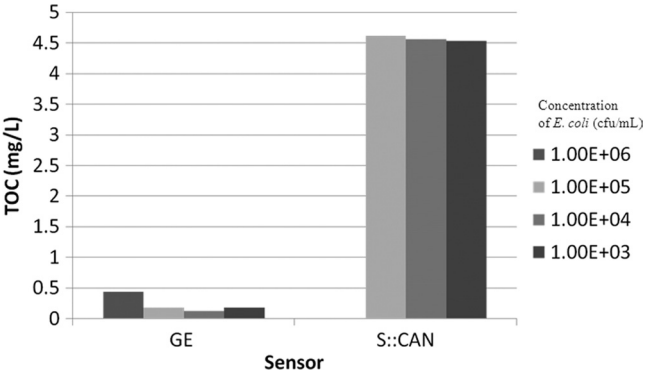

FIG. 4. Total organic carbon measured in tap water with an intrusion of washed *E. coli* at the Real Time Monitoring Laboratory. S::CAN did not generate data at 10^6 CFU/ml due to device detector saturation.

This work was supported by the University of Arizona National Science Foundation Water and Environmental Technology Center, the University of Arizona Water Sustainability Program, and the National Institute of Environmental Health Science-sponsored Southwest Environmental Health Sciences Center grant no. P30 ES06694.

REFERENCES

1. **Hall, J., et al.** 2007. On-line water quality parameters as indicators of distribution system contamination. J. AWWA **99:**66.
2. **Helbling, D. E., and J. M. Vanbriesen.** 2007. Free chlorine demand and cell survival of microbial suspensions. Water Res. **41:**4424–4434.
3. **Helbling, D. E., and J. M. Vanbriesen.** 2008. Continuous monitoring of residual chlorine concentrations in response to controlled microbial intrusions in a laboratory-scale distribution system. Water Res. **42:**3162–3172.
4. **Hobbie, J. E., R. J. Daley, and S. Jasper.** 1977. Use of Nuclepore filters for counting bacteria by fluorescence microscopy. Appl. Environ. Microbiol. **33:** 1225–1228.
5. **National Research Council.** 2004. A review of the EPA water security research and technical support action plan. Panel on water system security research. National Academies Press, Washington, DC.
6. **National Research Council.** 2006. Drinking water distribution systems: assessing and reducing risks. National Academies Press, Washington, DC.
7. **U.S. Environmental Protection Agency.** 2010. Detection of biological suspensions using online detectors in a drinking water distributions system simulator. EPA/600/R-10/005. U.S. Environmental Protection Agency, Security Research Center, Cincinnati, OH.