Real-time detection of microbial intrusion in potable water using advanced devices

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Abstract

The objective of this work was to evaluate the efficiency of online sensors in detecting biological contamination events in drinking water networks. An online sensor "S::CAN" integral with a 61 m laboratory scale distribution system (LDS), constructed at the University of Lille, were used to study the change in multiple parameters of tap water induced by injection of Escherichia coli cells under different concentrations from 10^5 UFC/ml to 10^8 UFC/ml. The magnitude of response for TOC, conductivity, free chlorine concentration and absorbance was proportional to injected cell concentration for a concentration exceeding 10^6 UFC/ml. The present study show that the S::CAN sensor detected *E.coli* cells and could be utilized as part of a contaminant warning Supervisory Control and Data System (SCADA), for real time monitoring intrusion events in water distribution systems.
Keywords: E.coli, contaminant, S::CAN, on-line monitoring.

1 Introduction

Access to water of a high quality through sustainable treatment and effective water distribution systems is essential to contemporary life in developed countries.

Traditionally, to monitor the water quality for microbial contamination, municipal water utilities are based on tests presence of indicator organisms (i.e. tests for fecal coliform or for *E. coli*) which requires up to 24 hours to get results (USEPA [1]), and remains unable to monitor pathogens that must be detected in real time.

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Consequently, distribution systems are considered relatively unprotected and vulnerable to intentional, natural or accidental contamination with microbial agents. The lack of real-time monitoring on the distribution systems potentially exposes the public to pathogenic microorganisms (Yang *et al.* [2]). Thus, there is a clear need to rapidly detect (and respond) to instances of accidental (or deliberate) contamination, due to the potentially severe consequences to public health (Storey *et al.* [3]).

The use of smart sensors built with a capacity to recognize and to diagnose, minute to minute the water quality disturbances, can monitor water quality through rapid detection of intentional intrusion or operational events, improving consequently the safety of water. When contamination events are recognized in real time, rapid response can minimize the impact of these incidents and limit the risk of adverse effects.

In this paper we present tests conducted in a pilot scale drinking water distribution system simulator (DSS) to evaluate the effectiveness of S::CAN for the detection of multiple density of microbial suspensions.

For this purpose, *E.coli* cells, indicators for drinking water quality assessment (Dawson and Sartory [4]) were injected into the DSS and the sensor responses were compared to a stable baseline values before injection.

2 Materials and methods

At the University of Lille, a laboratory-scale distribution system (LDS) is used to study the efficiency of online sensors in detecting microbial contamination events in drinking water networks.

Water used in the following experiment is delivered by the Eau Du Nord to the scientific city.

2.1 The laboratory scale network

The pilot used in this study is composed of 16 mm opaque double layer pipes (aluminum outside and plastic inside). The complete network diagram is shown in fig. 1;

A tank noted (1) of 40 L volume supplied the network by a centrifugal pump (3). A small tank noted (2) is used to inject substances into the system.

The water quality monitoring systems (S::CAN) is connected to the slot at the position (7), while the temperature is continuously monitored at the location (6).

The flow in the system can be manually controlled by using multiple valves in the circuit and measured with the flow meter (5).

In the present work, the distance between the injection septum of chemical or biological products and the monitoring systems is 41 m. During the experiments in open circuit, water is transmitted to an external drain (9) for the disinfection and subsequent disposal. After each experiment the system is emptied by opening the valve (8) and then disinfected by feeding the circuit from chlorine tank noted (10). Thereafter, the system is emptied, washed with water, and finally dried with compressed air to prevent the formation of biofilms.

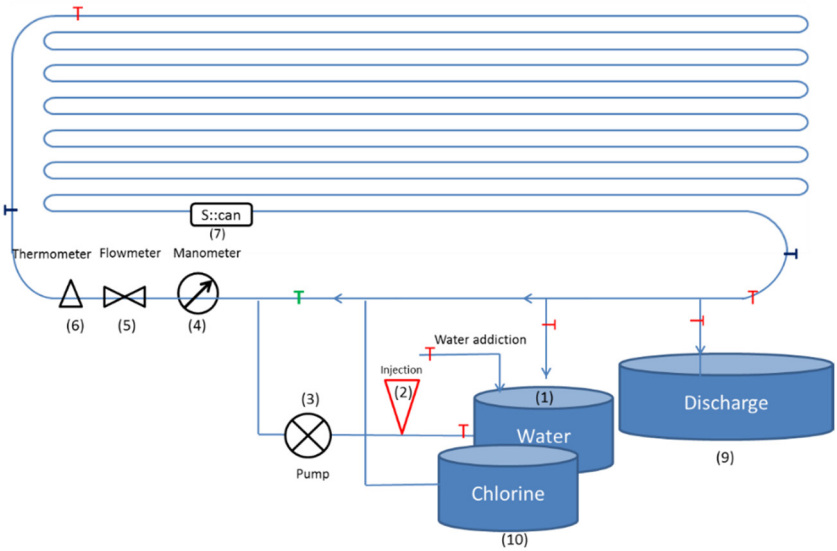

Figure 1: Schematic diagram of laboratory-scale distribution system.

Figure 2: Photo of the network at the laboratory scale.

2.2 S: CAN:spectro::lyse Technolo

S::CAN is a multi-parameter sensor that uses UV-visible spectroscopy to measure water quality. Once the UV spectrum of the non-contaminated water is

established by continuous measurement of water quality parameters, the contamination in the water can be detected as a deviation from the baseline or reference of the initial spectrum (Van den Broeke [5]).

The sensor provides spectral data between 200 and 700 nm with reference to pre-determined algorithms for several water quality parameters that detect changes of the baseline of the UV spectrum. Output measurements from the S::CAN analyzed for this study include turbidity, conductivity, free chlorine, total organic carbon (TOC), the pH and temperature.

2.3 Experimental protocol

The system is washed in an open circuit for 30 minutes, a baseline is determined by continuous measurement of tap water by S::CAN for 60 minutes.

Later, 2 liters of contaminants have been injected into the water system to analyze the sensor response to this injection.

Controlled injections of *E.coli* were performed under conditions provided in Table 1.

The distance between the injection of contaminants and the sensor sampling points is of 41m and the pressure in the system was of 2 bars. Therefore, under a flow of 2.8 L/min, the longest contact time available for any experiment was of *ca.* one minute.

Because of this physical constraint and to increase the contact time, the injected volume of *E. coli* cells was chosen to be 2 L.

To assess the sensitivity of the sensor, the injection was done in several concentrations of *E. coli* cells (10^5 CFU/ml, 10^6 CFU/ml, 10^7 CFU/ml and 10^8CFU/ml). For each concentration, the injection was repeated 3 times.

The system pressure was 2 bars. Injection times are as shown in Table 1.

Table 1: Conditions for *E.coli* injection into laboratory-scale distribution system.

Time	Flow (L/min)	Concentration (CFU/ml)
11 :24	2.8	10^5
11 :39	2.8	
11 :55	2.7	
12 :11	2.8	10^6
12 :27	2.7	
12 :47	2.6	
12 :57	2.8	10^7
13 :12	2.7	
13 :27	2.8	
13 :42	2.7	10^8
13 :57	2.8	
14 :12	2.8	

2.4 Bacterial culture and cell suspension preparation

The bacterial cells used for this study were *Escherichia coli* CIP 54127, stored at -80°C in Tryptic Soy Broth containing 40% (v/v) of glycerol (TSB; Biokar Diagnostics, France). Pre-culture was inoculated by 100 µl from frozen tube and grown in 5 mL of TSB at 37°C during 24 h. 1 mL of this preculture, containing $5x10^4$ CFU, was used to inoculate 50 ml of TSB medium in 500 mL sterile flasks for bacterial cultures. Cultures were then incubated at 37°C, under shaking condition (160 rpm), and bacterial cells were harvested in the late exponential phase (after 15 h) by centrifugation for 10 min at 3500 *g* (20°C). Bacteria were washed twice with 20 mL of potassium phosphate buffer (PB; 100 mM, pH 7) and finally resuspended in 20 mL of PB. To disperse cells, a sonication at 37 kHz was carried out for 5 min at 25°C (Elmasonic S60H, Elma, Germany). Subsequently, bacteria were resuspended in PB to a cell concentration of $1x10^8$ CFU/mL by adjusting the optical density to OD_{620nm}= 0.110 ± 0.005 (Ultrospec 1100 pro, GE Healthcare, formerly Amersham Biosciences, United Kingdom). Standardized cell suspensions were diluted in distilled water in order to make a cell concentration of 10^5, 10^6, 10^7, 10^8 CFU/mL for experiments.

2.5 Evaluation analysis of spiking experiment

For a concentration level equal and lower than 10^5 UFC /ml, the sensor has not showed significant consistent responses from the baseline values. Responses are visible from a concentration higher than 10^6 CFU. At this concentration, responses to spore inputs were significantly different from baseline values. This response is visible in the turbidity, TOC, color and UV254. These parameters respond to absorption/diffraction of light in the sample, therefore they all respond when the sample becomes less clear/changes color. Table 2 summarizes variation of absorbance, turbidity, color, total organic carbon and free chlorine the concentration after *E.coli* injections.

Table 2: Summary of mean variation due to *E.coli* injections.

Concentration CFU/ml	UV 254	Turb ISO – [FTU]	TOCeq – [mg/l]	Color T – [Hazen]	Free Chlorine
10^5 UFC /ml	1.752205	1.870783	0.8	–	0.238292
	-0.01887	0.019217	0	–	-0.00562
10^6 UFC /ml	1.724561	1.839221	0.8	–	0.310722
	0.333333	0.132779	0.133333	–	-0.07872
10^7 UFC /ml	1.740468	1.794031	0.8	–	0.229125
	0.424444	0.22745	0.166667	–	-0.08112
10^8 UFC /ml	1.733333	1.751333	0.8	0	0.135494
	2.966667	0.938333	1.366667	2.43	-0.08983

The followings graphs depict the change of absorbance, turbidity, TOC and free chlorine concentration measured by the sensors as a response of different concentrations of *E.coli*. It has been shown that the contaminant increases distinctly the absorbance, turbidity, and TOC and decreases the free chlorine concentrations.

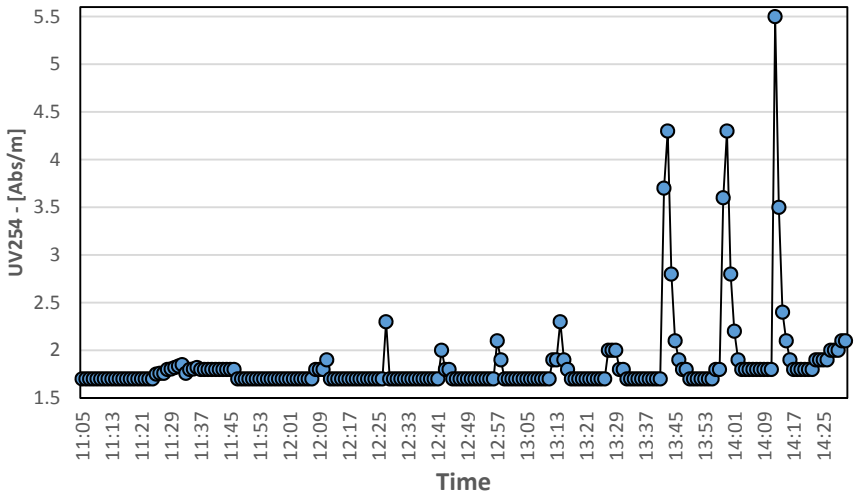

Figure 3: UV 254 Variation in response to *E.coli* injections.

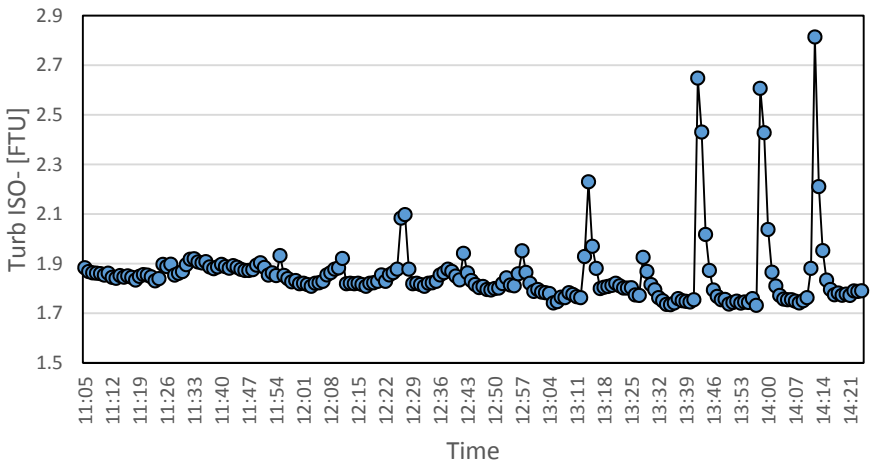

Figure 4: Turbidity variation in response to *E.coli* injections.

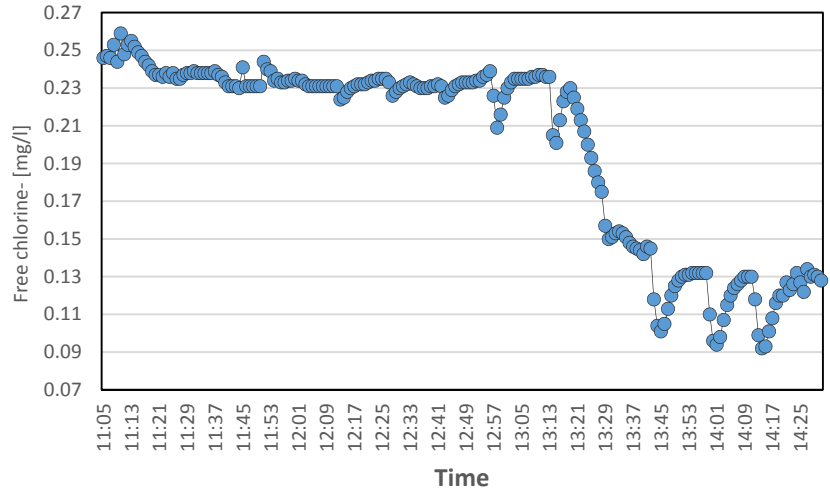

Figure 5: Free chlorine variation in response to *E.coli* injections.

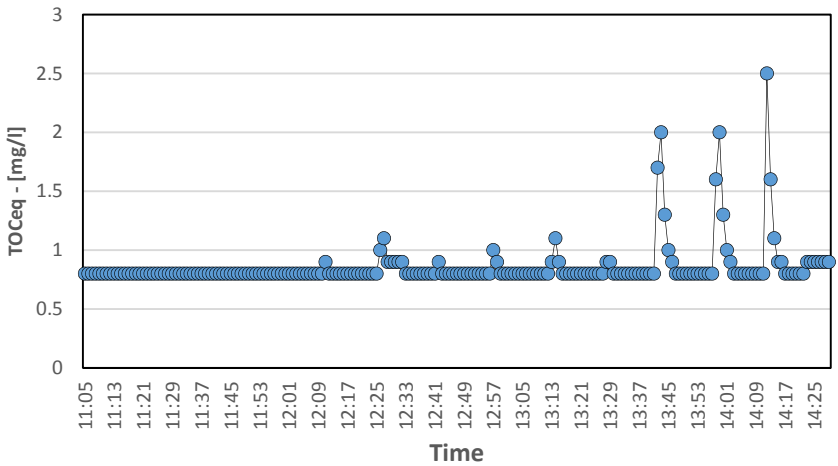

Figure 6: TOC variation in response to *E.coli* injections.

3 Conclusion

The data from this study evaluated the sensitivity of an UV-Visible spectroscopy based sensor (S::CAN) to detect microbial intrusions in real-time. The S::CAN sensor successfully detected *E.coli* in real time with a detection limit of 10^6 CFU/ml.

The results suggest that the S ::CAN could be used as part of a contaminant warning Supervisory Control and Data System (SCADA), for monitoring biological events in water distribution systems.

This study continues to test the sensor with other chemical and biological contaminants and for its comparison with other multi-parameter water quality sensors.

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