Development of a sensor for the detection of Escherichia coli in brackish waters

Mancuso Monique1, Grossi Marco2, Rappazzo Alessandro Ciro1, Zaccone Renata1, Caruso Gabriella1, Riccò Bruno2, Bergamasco Alessandro1

1Institute for Coastal Marine Environment (IAMC-CNR)-Section of Messina, Spianata S. Raineri 86, 98122, Italy

2Department of Electronic Engineering (D.E.I.S.) Bologna-University of Bologna, Via Zamboni 33, 40126 Bologna, Italy

ARTICLE INFO

Article history:
Received 2 Dec 2015
Accepted 5 Jan 2016
Received in revised form 18 Dec 2015
Available online 27 Feb 2016

Keywords:
Monitoring
Waters
Escherichia coli
Portable sensor
Fecal pollution
Environment

ABSTRACT

Monitoring of bacterial pathogens is important for marine environmental protection, because the presence of these microorganisms can be a serious risk for human health. For this reason, a portable sensor implemented as an electronic embedded system featuring disposable measurement cells was used to evaluate the ability and sensitivity of detection of Escherichia coli (E. coli) as an indicator of fecal pollution in transitional environments and a water sample added with E. coli (10^2 CFU/mL) was assayed. The first result obtained from the laboratory experiment seems promising for the determination of E. coli in environmental samples, though further improvements will be needed for the field application of this sensor in marine and brackish waters.

Enzymatic and immunological methods have been proven to be highly specific for E. coli detection, though a detection limit of 10^2 CFU/100 mL was stated in previous studies[5-10].

Impedentiometric methods offer another strategy for bacterial pathogen detection[11,12]. A portable sensor implemented as an electronic embedded system featuring disposable measurement cells already described by Grossi et al. was used with the aim of evaluating the suitability and the sensitivity of this device in the detection of E. coli contamination in transitional waters[11,12].

2. Materials and methods

The experimental trials were carried out using a strain of E. coli which was spread on Mc Conkey agar (Liofilchem) incubated at 37 °C for 24 h. After this period, the strain was suspended into lactose broth and incubated at 37 °C for 24 h. The growth curve was followed by 600 nm optical density.

Preliminary tests were carried out with the concentrations of E. coli in lactose broth (Liofilchem) ranging from 10^8 to 10^8 CFU/mL, as estimated by a McFarland equivalent turbidity standard 0.5 (corresponding to 10^4 CFU/mL).

In a successive step, tests were performed with E. coli and with lake water (34 pressure) and without E. coli. This last assay was used as a negative control to check the absence of any instrument background signal.

The scheme of the embedded biosensor system and the electrical model of the system electrodes-electrolyte were shown in Figure 1A, B. A picture of the embedded biosensor system.
was shown in Figure 1C. The sample under test (diluted in the enriching growth medium) was stored in a 50 mL polypropylene Falcon vial (hereafter the sensor) modified to house a couple of stainless steel electrodes to measure the electrical parameters of the sample during the bacterial growth.

The measure was carried out by stimulating the sensor with a sine-wave voltage signal of 200 Hz frequency and 100 mV amplitude. Measured data were acquired by a laptop PC for data display and logging. From an electrical point of view, the sensor could be modeled with the series of a resistance $R_s$ (accounting for the sample bulk resistance) and a capacitance $C_s$ (accounting for the electrodes-electrolyte capacitive interface). As discussed in Grossi et al.[12], the monitored electrical parameter was almost constant as long as the bacterial concentration lower than $10^7$ CFU/mL, while when this threshold was exceeded, the parameter deviated from the baseline value. The detect time (DT), defined as the time needed for the monitored electrical parameter to deviate from its baseline value, was known to be a linear function of the logarithm of the sample bacterial concentration. The first trial was performed using a mixture of lactose broth and lake water (1:1) without *E. coli*, while the second test consisted of sterile broth, lake water and a low concentration ($10^2$ CFU/mL) of *E. coli*. The Falcon tube was incubated at 37 °C according to the analytical protocol by Grossi et al.[12]. After 24 h, measurements of optical density were performed and 100 µL of broth were collected and spread on Mc Conkey agar for the bacterial count.

3. Results

Water samples with concentrations of *E. coli* ranging from $10^6$ to $10^2$ CFU/mL were preliminarily assayed to estimate the detection limit of the instrument as shown by Grossi et al.[12], where both the resistance $R_s$ and the capacitance $C_s$ were monitored in each assay.

The analysis of the brackish water samples added with *E. coli* $10^2$ CFU/mL showed that the curves of $C_s$ were characterized by lower noise, higher repeatability and allowed a more accurate estimation of the bacterial concentration than the $R_s$ curves. This

![Figure 1](image1.png)

**Figure 1.** A: schematic of the biosensor system; B: electrical model of the sensor; C: picture of the biosensor system.

![Figure 2](image2.png)

**Figure 2.** Capacitance curves (referred to the baseline value) vs. time in the case of a sterile sample and a sample inoculated with $10^2$ CFU/mL of *E. coli*. 
could be due to the electrical properties of the sample that the brackish water was characterized by a relatively high salinity and thus this high ionic content could interfere with the ions produced by bacterial metabolism, making difficult to detect variations in the sample bulk resistance (Rs). In the case of Cs, instead, only the electrodes-electrolyte interface was involved and thus the measure was less affected by the sample ionic content. Only data from the Cs curves were presented. In Figure 2, the Cs values (referred to the corresponding baseline values) measured at time intervals of 5 min were plotted vs. time in the case of a sterile sample and a sample inoculated with a concentration of 10^7 CFU/mL of E. coli. While in the case of the sterile sample, the Cs curve presented only small variations over time, since the bacterial concentration never reached the threshold concentration of 10^7 CFU/mL; in the case of the sample with 10^7 CFU/mL, there was a steep increase of Cs after 230 min (the DT value of this sample).

Measuring the DT of a set of samples with different initial bacterial contamination, a calibration line could be calculated, thus allowing to estimate the bacterial concentration of sample from the measured DT.

There were several methods to detect E. coli such as, immune and enzymatic techniques that all these methods needed to carry the samples to the laboratory to perform the analysis[2-6].

4. Discussion

In this work, we tested a system that can be mounted on a buoy enabling the automatic in situ detection of E. coli contamination. Future steps of development will include the improved design of a tailored microcontroller to manage multiple samples and send the results directly to the laboratory personal computer (Asus) to simplify the operations and reduce execution time analysis.

The advantages of this sensor with respect to the traditional method can be summarized as follows: 1) the system can be easily managed by a microcontroller, mounted on a buoy and placed where needed; 2) the system can be modified with the addition of a mini-rosette to enable multiple samplings (per period or day); 3) this system avoids shipping samples to the laboratory, so time and money can be saved; 4) the system can be linked to the web and therefore, the data can be sent and made available in real time and 5) finally, it is not necessary cultivation of the microorganism or performs count on culture medium. On the other hand, there are some critical issues to be solved: 1) the system might be too sensitive to temperature and particularly to salinity changes that can happen in transitional waters; 2) if the concentration of E. coli is too low, the system needs more time to reach the detection threshold. In fact, in the examined lakes, the concentrations of E. coli are very low, reaching a maximum of 10^7 CFU/mL (to date, the European directive 2006/113 and the Italian D.Lgs. 2006/152 arts.87-88 set up the threshold value of 3 x 10^5 CFU/mL inside the shellfish).

To improve the performances of the portable biosensor and make it suitable in operational monitoring of coastal and transitional waters contamination, further analyses will be carried out to face the following aspects which we believe crucial: (i) to lower the detection threshold (currently set at 10^7 CFU/mL) to 10^6 CFU/mL (this would mean to achieve a shorter measurement time, saving to 2 h); (ii) to modify the instrument to be mounted on the buoy; (iii) to find a better method/system to sterilize the cuvette on the buoy (i.e. by UV rays) or adopt a multiple carrier of disposable cuvettes.

This first result obtained from the laboratory experiments with the portable device seems to be promising for the determination of E. coli in natural environmental samples, though further assays will be performed with a range of concentration from 10^5 to 10^7 CFU/mL to set the detection limit of brackish water samples of this instrument. Moreover, further improvement will be needed for the field application of this sensor in marine and brackish waters.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The research has been carried out within the activities of the nationally-funded project SSD-Pesca “decision support system for sustainable fisheries management in the regions of Southern Italy” (Workpackage 1-CNR-IAMC Messina) (Law 191, December 23, 2009, article 44) aiming at the development and implementation of innovative technologies, instruments and systems to foster a responsible development of the fishing activities in Southern Italy.

References

[1] Zaccone R, Mancuso M, Modica A, Zampino D. Microbiological indicators for aquaculture impact in Mar Piccolo (Taranto, Italy). Aquac Int 2005; 13: 167-73.
[2] Zaccone R, Azzaro M, Azzaro F, Bergamasco A, Caruso G, Leonardi M, et al. Seasonal dynamics of prokaryotic abundance and activities in relation to environmental parameters in a transitional aquatic ecosystem (Cape Peloro, Italy). Microb Ecol 2014; 67: 45-56.
[3] Caruso G, Leonardi M, Monticelli LS, Decembrini F, Azzaro F, Crisaì E, et al. Assessment of the ecological status of transitional waters in Sicily (Italy): first characterisation and classification according to a multiparametric approach. Mar Pollut Bull 2010; 60: 1682-90.
[4] Caruso G. Microbes and their use as indicators of pollution. J Pollut Eff Control 2013; doi: 10.4172/2375-4397.1000e102.
[5] Caruso G, Crisaì E, Mancuso M. Development of an enzyme assay for rapid assessment of Escherichia coli in seawaters. J Appl Microbiol 2002; 93: 548-56.
[6] Caruso G, Crisaì E, Mancuso M. Immunofluorescence detection of Escherichia coli in seawater: a comparison of various commercial antiserum. J Immunoassay Immunochem 2002; 23: 479-96.
[7] Caruso G, Denaro R, Genovese M, Giuliano L, Mancuso M, Yakimov M. New methodological strategies for detecting bacterial indicators. Chem Ecol 2004; 20: 167-81.
[8] Caruso G, Mancuso M, Crisaì E. Combined fluorescent antibody assay and viability staining for the assessment of the physiological states of Escherichia coli in seawaters. J Appl Microbiol 2003; 95: 225-33.
[9] Caruso G, De Pasquale F, Mancuso M, Zampino D, Crisaì E. Fluorescent antibody-viability staining and beta-glucuronidase assay as rapid methods for monitoring Escherichia coli viability in coastal marine waters. J Immunoassay Immunochem 2006; 27: 1-13.
[10] Caruso G, Crisaì E, Caruso R, Zappalà G. Advances in marine bacterial pollution monitoring. In: Kuriladze GV, editors. Environmental microbiology research trends. Hauppauge N.Y.: NOVA Science Publishers; 2008. p. 273-87.
[11] Grossi M, Lanzoni M, Pompei A, Lazzarini R, Matteuzzi D, Riccò B. An embedded portable biosensor system for bacterial concentration detection. Biosens Bioelectron 2010; 26: 983-90.
[12] Grossi M, Lazzarini R, Lanzoni M, Pompei A, Matteuzzi D, Riccò B. A portable sensor with disposable electrodes for water bacterial quality assessment. IEEE Sens J 2013; 13: 1775-82.