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

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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.

1. Introduction

Transitional water systems, such as lagoons, estuaries and coastal lakes, are very complex ecosystems located at the interface between land and sea, characterized by confined circulation and weak hydrodynamism, shallow depth and strong variations in temperature and light regimes, high productivity, high potential biodiversity and high vulnerability to anthropic pressure[1,2].

The assessment of the health status of these water systems is a major issue for accurate management and preservation of their integrity[3]. The availability of standardized buoy systems with hosting several sensors, following temporal changes, could provide accurate description of water contamination occurring over time in these particular ecosystems. Faecal pollution is generally detected through the estimation of the presence and abundance in the waters of the microorganism [*Escherichia coli* (*E. coli*)], which represents one of the most significant indicators of sewage contamination[4]. Several methods are conventionally used for environmental monitoring of *E. coli* and can be summarized as cultural, biochemical and molecular methods. 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 10^6 to 10^2 CFU/mL, as estimated by a McFarland equivalent turbidity standard 0.5 (corresponding to 10^8 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

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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 Rs (accounting for the sample bulk resistance) and a capacitance Cs (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 preliminarly assayed to estimate the detection limit of the instrument as shown by Grossi *et al*.[12], where both the resistance Rs and the capacitance Cs 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 Cs were characterized by lower noise, higher repeatability and allowed a more accurate estimation of the bacterial concentration than the Rs curves. This

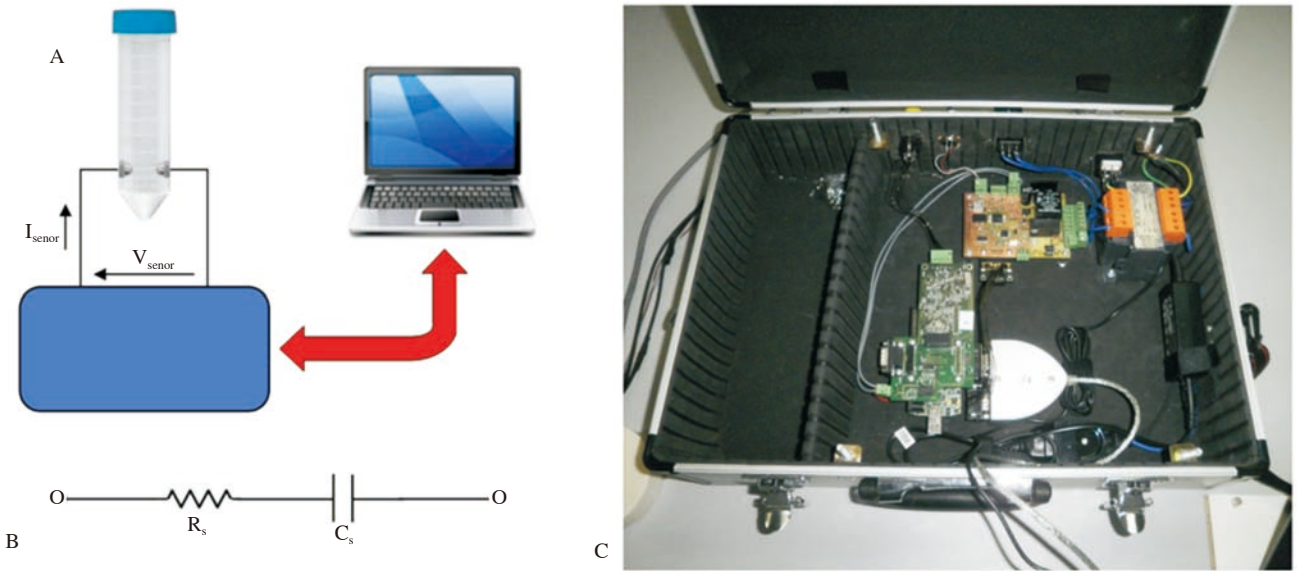

Figure 1. A: schematic of the biosensor system; B: electrical model of the sensor; C: picture of the biosensor system.

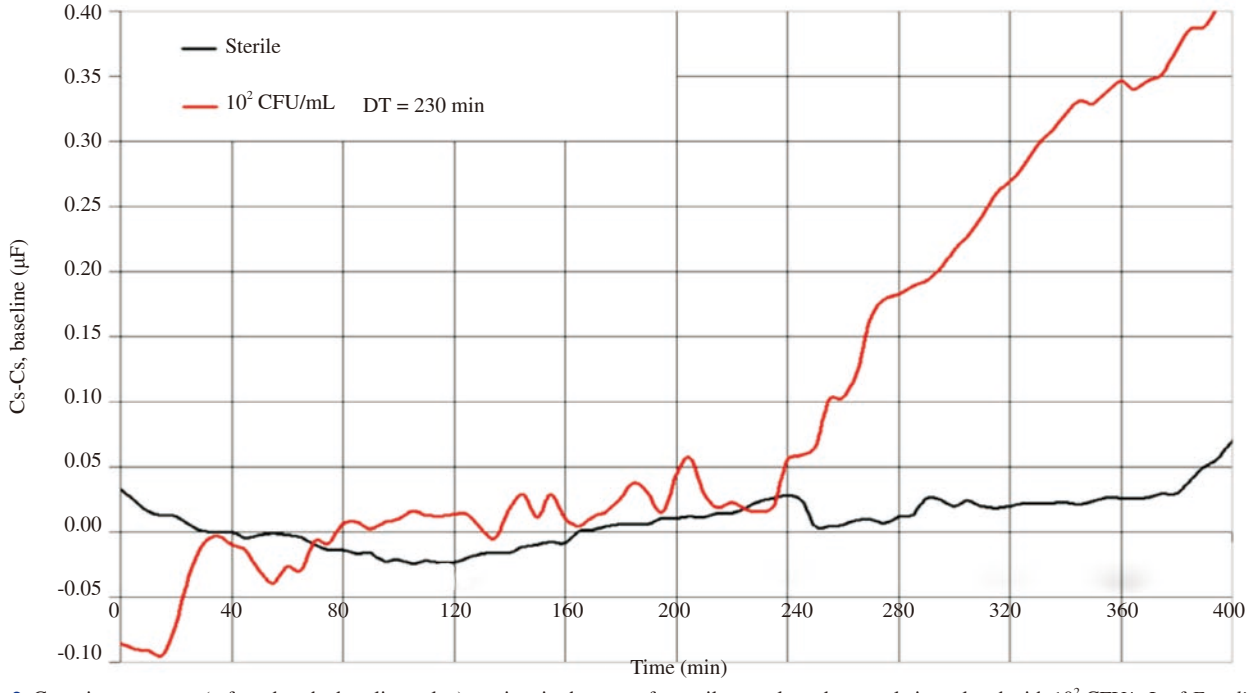

Figure 2. Capacitance curves (referred to the baseline value) *vs*. time in the case of a sterile sample and a sample inoculated with 10^2 CFU/mL of *E. coli*.

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 bacteria 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 10^2 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 10^2 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 10^2 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 3×10^2 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 10^5 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 10^6 to 10^1 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

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