Bacterial concentration detection using a portable embedded sensor system for environmental monitoring

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Abstract— **The detection of bacterial concentration is important in different fields since high microbial contamination or the presence of particular pathogens can seriously endanger human health. The reference technique to measure bacterial concentration is Standard Plate Count (SPC) that, however, has long response times (24 to 72 hours) and is not suitable for automatic implementation. This paper presents a portable embedded system for bacterial concentration measurement based on Impedance Microbiology that is suitable for in-situ measurements and does not require trained personnel. The system has been tested with samples inoculated with different concentrations of *Escherichia coli* and its response correlates very well ($R^2 = 0.9185$) with results from SPC.**

Keywords—**sensor; impedance; bacteria; embedded system; measurement; environmental monitoring.**

I. INTRODUCTION

Bacterial concentration detection is a very important issue in different fields [1], such as food quality assurance [2, 3], clinical analysis [4] and environmental monitoring [5]. Microbial monitoring is important both to assure that the total bacterial concentration does not exceed thresholds set by national and international regulations, and to guarantee the absence of particular pathogens, such as *Salmonella typhimurium* and *Escerichia coli* O157:H7, representing a threat to human health even at very low concentrations.

The reference technique to measure bacterial concentration is Standard Plate Count (SPC) [6], a reliable and accurate method that, however, features long response times (usually from 24 to 72 hours) and is still largely carried out manually by skilled personnel in lab environment.

Alternative techniques for bacterial concentration measurements have been proposed in the literature in recent years. In the turbidimetric method [7], for instance, the optical absorbance at a particular wavelenght (usually 602 nm) is measured and used to estimate bacterial concentration. The main drawback of such a method is that only non-opaque samples can be tested and samples without bacterial contamination must also be available as control. Instead, bioluminescence techniques [8] exploit the property of some bacterial species to emit photons as by-product of their reactions, but the sensitivity and response time is strongly affected by the particular microbial strain. Impedance biosensors [9, 10] use particular bio-receptors immobilized on sensing electrodes that can bind to the target bacterial strain, modifying the sensor electrical parameters. This approach allows selective measurements of bacterial concentration with high sensitivity and low response time. However so far the stability of bio-receptor immobilization and the presence of non-specific reactions have prevented the commercial success of actual devices. Flow cytometry, used in commercial instruments such as Bactoscan by Foss Electric, allows to measure total bacterial concentration with response times as low as 20 minutes. However these instruments are expensive and can be afforded only by big organizations.

An interesting alternative to the previous methods is Impedance Microbiology (IM), a relatively old technique that estimates bacterial concentration by measuring electrical changes in the growth medium induced by bacterial metabolism.

In IM the Sample Under Test (SUT) (possibly diluted in an enriching medium) is placed in direct contact with a couple of electrodes at a temperature favoring bacterial growth and the SUT electrical parameters are measured at regular time intervals. Since bacterial metabolism transforms uncharged or weakly charged compounds present in the growth medium in highly charged ones, the SUT electrical parameters change with the bacterial growth. In practice, they remain almost constant (at a baseline value) until the bacterial concentration reaches a critical threshold (in the order of 10^7 colony forming units (CFU)/ml), when they start to significantly deviate from the baseline. The time required for such a deviation to occur (i.e. the time needed for the bacterial population to grow up to a concentration of 10^7 CFU/ml) is denoted Detect Time (DT) and is a linear function of the logarithm of the SUT bacterial concentration [11]. Thus, the DT can be used to estimate the initial SUT bacterial concentration once the system has been properly calibrated for the particular sample type and bacterial strain.

IM can be used as a quick alternative to SPC to measure bacterial concentration with time response in the range 2 to 12 hours (depending on the SUT level of contamination). Furthermore, the IM technique can be implemented in the form
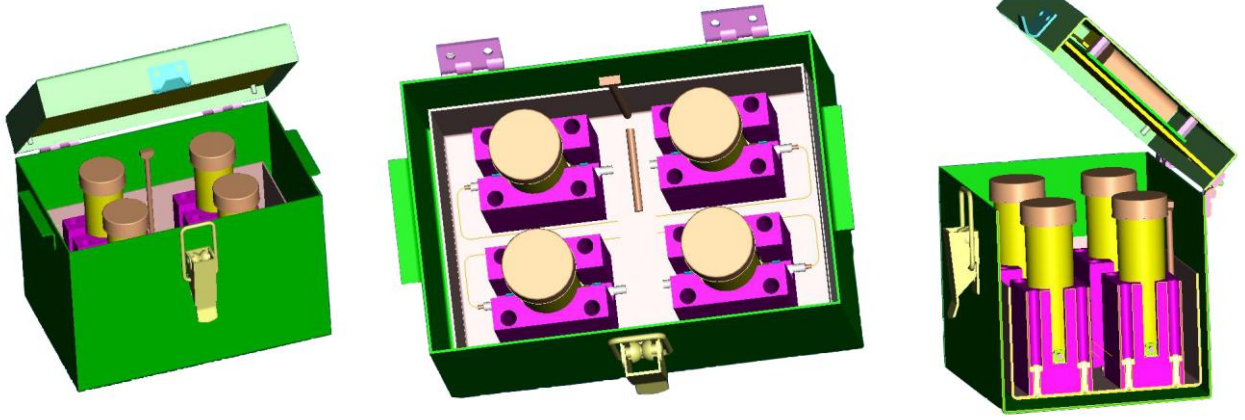
Fig. 1. Pictures of the portable sensor system.

of devices embedded in an industrial machine or portable instruments for in-situ measurements.

IM has been successfully tested for bacterial concentration measurements in various types of samples (ice-cream [12, 13], meat [14], vegetables [15], raw-milk [16], fresh water [17], beer [18], *Escherichia coli* in human urine samples [19]) and to test the efficiency of various antibiotics and chemical preservatives [20, 21].

This paper presents a portable bio-sensor that measures bacterial concentration by IM. The system features a thermal chamber to store the SUTs at the target temperature, two ad-hoc designed electronic boards, a graphic TFT LCD display, four buttons for user interaction and a mini USB port to tranfer the measured data to a PC for filing and further processing. The system can be used everywhere and by anyone for in-situ bacterial concentration measurements.

II. EXPERIMENTAL DESIGN

The proposed system is shown in Fig. 1. It features a 26 x 18 x 25 cm, thermally isolated aluminium chamber where up to four sensors (50ml polypropilene vials modified to accommodate a couple of stainless steel electrodes) are hosted. The heating system is realized by four power resistances (50W 1.2Ω), placed at the bottom of the chamber, that regulate the inner temperature (T) by Joule effect. The top cover of the system hosts two ad hoc designed electronic boards (one for the thermoregulation the other for the electrical measurements), a graphic TFT LCD (2.83 inches, 320x240, Easy TFT Board by MikroElektronika) and four buttons for user interaction. A mini-USB port for serial data transfer to a PC is also provided.

The schematic of the system circuit is presented in Fig. 2 (a), (b) and (c). The thermoregulation board, shown in Fig. 2 (b), is based on the microcontroller STM32F103 by ST Microelectronics that drives the power resistances using a PWM signal and a FODM1008R2 octocoupler (Fairchild) to isolate the low-power section from the high-power section of the board. The target temperature T inside the chamber is regulated using a PID algorithm (updated every 1 sec) that modulates the duty cycle of the PWM signal (PWM_{DC}) according to the formula:

$$PWM_{DC,k} = P_k + I_k + D_k \quad , \tag{1}$$

where P_k, I_k, and D_k are the proportional, integral and derivative components at time k defined as:

$$P_k = K_P \times \left(T^* - T_k\right) \quad , \tag{2}$$

$$I_k = I_{k-1} + K_I \times \left(T^* - T_k\right) \quad , \tag{3}$$

$$D_k = K_D \times \left(T_{k-1} - T_k\right) . \tag{4}$$

Here: T_k is the temperature at time k (measured using a 10kΩ NTC thermistor inside the chamber), T^* the target temperature, K_P, K_I and K_D the PID parameters.

Figs. 3 (a) and (b) show the chamber temperature and PWM duty cycle vs. time for three different sets of PID parameters. The target temperature T^* has been set to 37°C. Higher values of K_I result excessive oscillations around the target point, while the derivative parameter K_D has almost no influence in achieving stable and accurate temperature. The PID parameters have been set as $K_P = 2$, $K_I = 0.003$, $K_D = 0$. With the appropriate choice of control parameters, the temperature reaches the target value of 37°C in about 30 minutes, with further oscillations smaller than 0.2°C.

The board devoted to the measurements is based on the microcontroller STM32L152 by ST Microelectronics, essentially sketched in Fig. 2 (c).

The SUT impedance (real and imaginary components) is calculated by stimulating the sensor electrodes with a sine-wave test voltage $V_{IN}(t)$ and measuring the current $I_{IN}(t)$ through the electrodes at three different frequencies (100Hz, 500Hz and 1kHz). This technique is called Electrical Impedance Spectroscopy (EIS) and is widely used as a sensing method for different applications such as: quality analysis in food products [22-26], human body composition monitoring [27-29], detection of corrosion in metal parts exposed to acid electrolytes [30, 31], measurement of oil concentration in water [32].

The input test voltage:

$$V_{IN}(t) = V_{M,IN} \times \sin(2\pi f t) + V_{DC} \tag{5}$$

is generated using a DAC channel of the microcontroller (DAC_1) with $V_{DC} = 1.65$V. $V_{M,IN}$ can be adjusted by
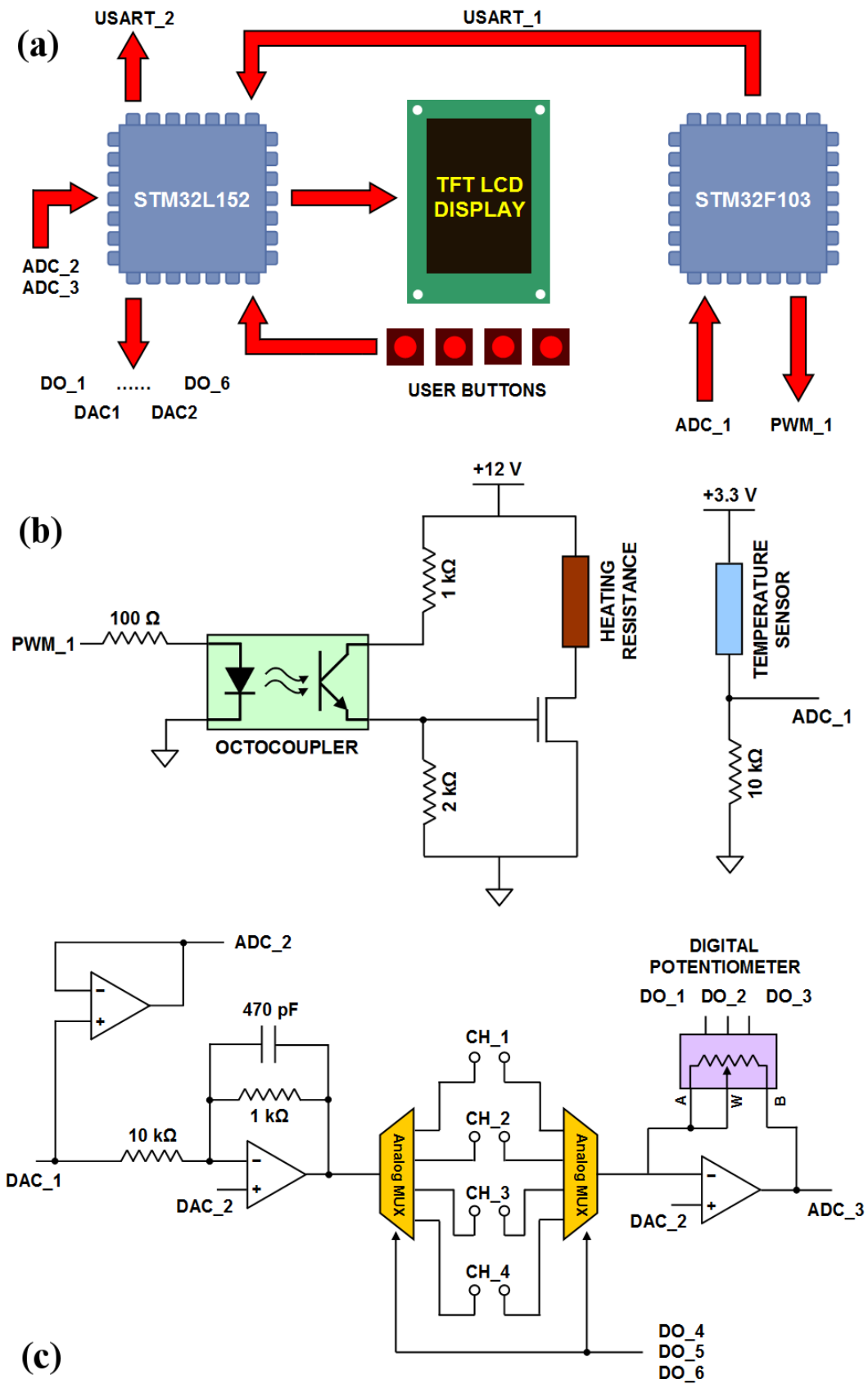
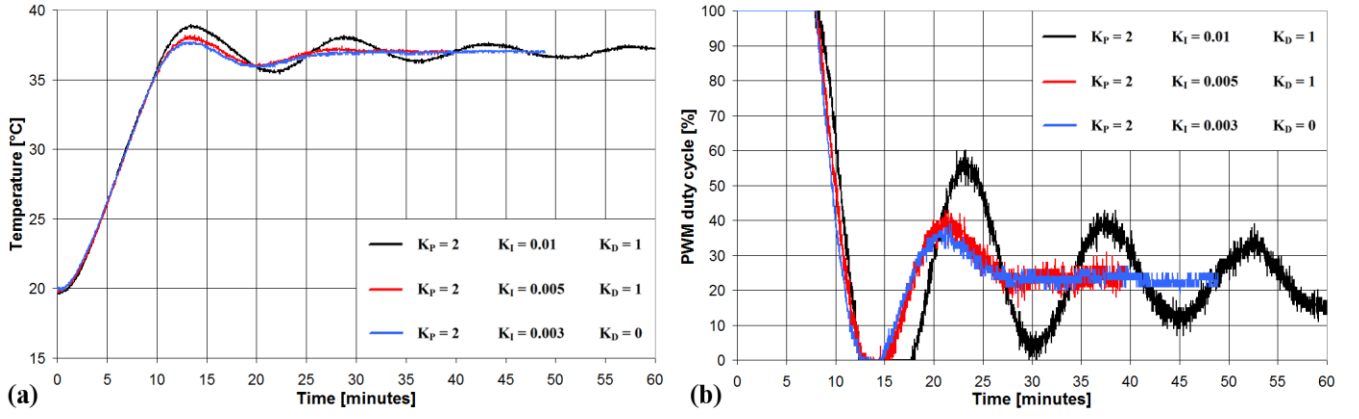Fig. 2. Schematic of the portable sensor system.

Fig. 3. Measured temperature (a) and duty cycle of the PWM signal used to power the heating resistances (b) vs. time for three different sets of PID parameters.

software between 10mV and 100mV. $I_{IN}(t)$ is fed to a current to voltage (I/V) converter that generates a voltage

$$V_{OUT}(t) = V_{M,OUT} \times \sin(2\pi ft + \varphi) + V_{DC} \qquad (6)$$

proportional to $I_{IN}(t)$. The I/V converter feedback resistor is a 10kΩ MCP4131 digital potentiometer, controlled by the microcontroller SPI interface to allow measuring a wide range of impedances. Two analog multiplexers (ADG804YRMZ) are used to selectively apply the test signal to one of the four SUTs that can be tested for each assay. The voltage signals $V_{IN}(t)$ and $V_{OUT}(t)$ are acquired by the microcontroller using two ADC channels and the signal parameters ($V_{M,IN}$, $V_{M,OUT}$ and φ) are calculated using the algorithm discussed in [33].

The electrical circuit used to model the SUT, shown in Fig. 4 (a), consists of the series of a resistance R_s, accounting for the bulk medium electrical conductivity, and of a Constant Phase Element (CPE) impedance Z_Q, accounting for the capacitive electrode-electrolyte interface. Z_Q can be described as:

$$Z_Q = \frac{1}{Q \times (j2\pi f)^\alpha} \quad , \qquad (7)$$

where: Q is the capacitive component and α an empirical parameter accounting for the non-ideal behavior of CPE (if α = 1 CPE becomes an ideal capacitance).

As the bacteria metabolic activity increases, R_s decreases while Q increases. Fig. 4 (b) shows the case of R_s: after an initial lag time, the SUT bacterial population begins to increase and when it reaches the critical threshold of 10^7 CFU/ml the R_s begins to deviate from its baseline value. Once this happens the microcontroller calculates the DT, that is used to estimate the initial bacterial concentration.

III. RESULTS AND DISCUSSION

The portable system discussed in Section 2 has been tested using a laboratory cultured strain of *Escherichia coli*. SUTs featuring different bacterial concentrations were created by inoculating the cultured strain (diluted in distilled water) into the sterile growth medium.
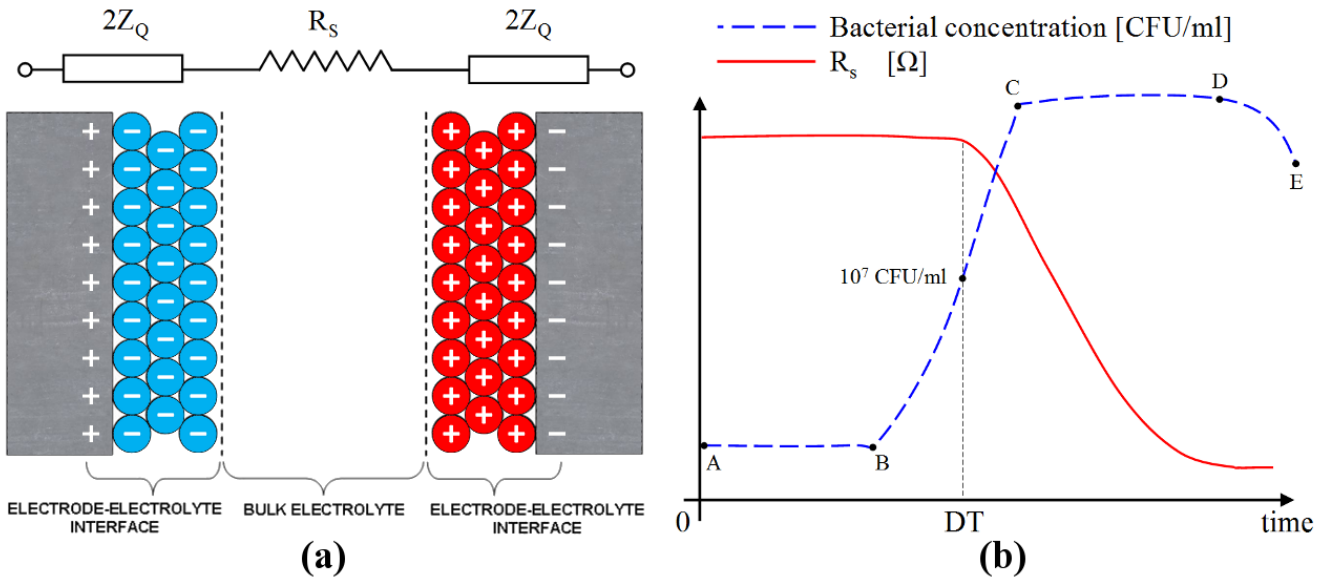

Fig. 4. (a) Electrical model for the electrode-electrolyte system; (b) resistance curve vs time for a sample featuring a growing bacterial population.
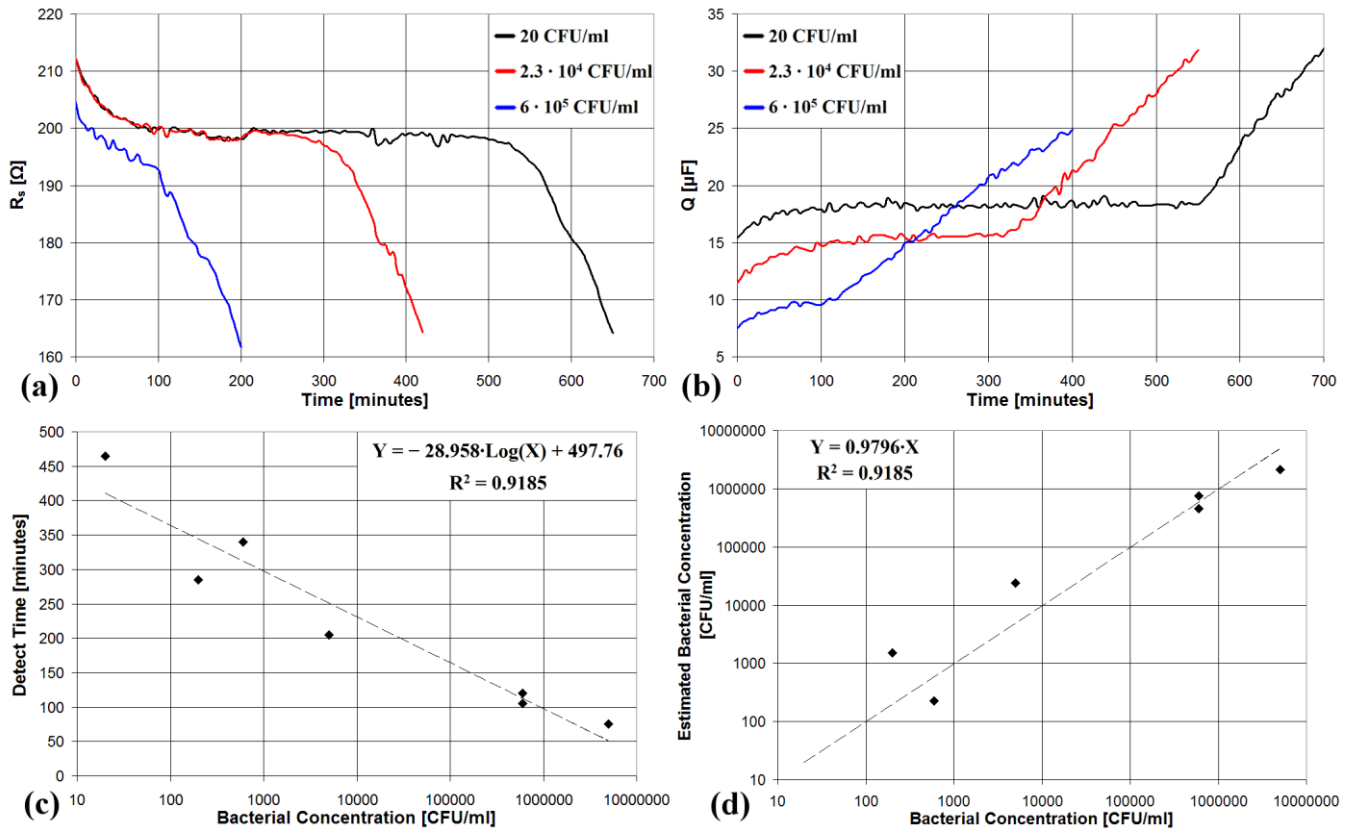
Fig. 5. Resistance (a) and capacitance (b) curves vs time for samples featuring different values of bacterial concentration. Calculated Detect Time (c) and estimated bacterial concentration (d) plotted vs bacterial concentration determined by SPC for all tested samples.

Two different growth media have been used: Luria Bertani (LB) broth featuring Tryptone 10g, Yeast Extract 5g, NaCl 10g (in 1 litre of distilled water) and Modified Luria Bertani (MLB) broth, prepared with the same ingredients of LB but without NaCl.

A total of 7 samples were tested in duplicate for both growth media and the DT measured for both electrical parameters (R_s and Q).

The results have shown that, when using MLB, both R_s and Q can be used to reliably estimate the bacterial concentration. Instead, when using LB as growth medium, only Q can be used to estimate bacterial concentration since R_s exhibits irregular curves over time and the corresponding DT can not be reliably calculated. This can be explained by the fact that the much higher ionic content of LB (compared to MLB) produces higher electrical conductivity of the medium hindering the increase due to bacterial metabolism, while the capacitance variations, mainly due to the electrode-electrolyte interface, are much less affected by the medium conductivity.

Figs. 5 (a) and (b) show the measured R_s and Q vs. time for assays carried out using MLB and different concentrations of *Escherichia coli*. As can be seen higher bacterial concentration results in lower values for the calculated DT for both R_s and Q. For example, in the case of R_s, the calculated DT is 535 min for a concentration of 20 CFU/ml, 310 min for a concentration of $2.3 \cdot 10^4$ CFU/ml and 115 min for a concentration of $6 \cdot 10^5$ CFU/ml.

Fig. 5 (c) shows the scatter plot for the calculated values of DT vs. the SUT bacterial concentration determined by SPC in the case of growth in LB and DT calculated by Q. As can be seen, a linear correlation exists between the calculated DT and the logarithm of the SUT bacterial concentration with a coefficient of determination $R^2 = 0.9185$. Fig. 5 (d) shows the estimated bacterial concentration calculated from the calibration line of Fig. 5 (c) vs. the SUT bacterial concentration determined by SPC.

The scatter plots were built for all the three conditions: (i) growth in LB and DT calculated by Q; (ii) growth in MLB and DT calculated by R_s; (iii) growth in MLB and DT calculated by Q. The calculated coefficient of determination is 0.9185, 0.8926 and 0.9196 for case (i), (ii) and (iii) respectively. The calculated slope of the linear regression line is −28.958, −35.353 and −36.611 for case (i), (ii) and (iii) respectively.

These results show how the bacterial concentration estimation obtained with the Q curves gives better correlation (i.e. higher R^2) with SPC than if R_s curves are used. Moreover, the use of LB as growth medium results in shorter response time (i.e. lower absolute values for the linear regression line slope) than in the case of MLB due to the presence of NaCl.

IV. CONCLUSIONS

A portable sensor system that measures bacterial concentration exploiting Impedance Microbiology has been

presented. The system features a heating chamber hosting up to four samples, two ad-hoc designed electronic boards, a TFT LCD and a mini-USB port for data transfer to a PC.

The system has been tested with different concentrations of *Escherichia coli* and the results have shown a very good correlation with the reference technique.

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