Automatic Evaluation of Water Quality Based on High Speed Networking

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***Abstract*— Bacterial screening is very important in water environmental monitoring, because the presence of dangerous pathogens can seriously endanger human health. Microbial concentration detection is performed by standard plate count technique, which is reliable but is characterized by long response time and is not suitable to be implemented in automatic form. Based on impedance measurements, this paper presents a portable sensor implemented as an electronic embedded sys-tem featuring disposable measurement cells, which is suitable of measuring bacterial concentration in water samples. The system provides a much faster response than standard technique (3-12 h depending on the contamination level versus 24-72 h of the standard technique) and can be used for an *in situ* microbial test rather than taking samples to a laboratory for analysis. Water samples from different sources (such as rivers, wastewaters, watercourses) are tested using the presented system. Enriched medium is added to the sample to favor bacterial growth. Three different media are tested (Lauria Bertani, Mc Conkey Broth, and Lactose Broth) and data are compared with microbial growth rate and selectivity toward bacterial group (e.g., coliforms). The obtained experimental data show good correlation with the plate count technique.**

***Index Terms*— Bacteria, coliforms, disposable electrodes, embedded system, impedance, portable sensor.**

I. Introduction

THE detection of water contaminants is of primary impor-tance in environmental monitoring since the presence of pathogens can seriously endanger human health [1]. This applies to drinking water as well as to river and seawater and wastewater.

Drinking water must be bacterial free and, to guarantee this, it undergoes different sterilization processes [2] (such as chlorination) to eliminate dangerous bacterial concentration.

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However, contamination could occasionally occur after these treatments during the distribution process [3]: thus after each treatment step water microbial concentration must be regularly monitored.

Rivers and seawaters must also be screened for pathogens and, although microbial concentration limit is not as strict as in the case of drinking water, bacterial concentration should be low enough as to not represent a threat to human health.

Industry wastewaters must also be screened for pathogens since they end up in public waters and contribute to envi-ronmental contamination. This in particular applies to food industries, such as water used in meat treatment plants, where animal carcasses can seriously contaminate the water resulting from the process.

Water microbial screening is regulated by national and international regulations [4] and is aimed at detecting the presence of dangerous contaminants. Usually this is obtained by screening the sample for microorganisms that are related to faecal contamination since, from a statistical standpoint, these present a good correlation with the presence of pathogens. Traditionally, coliforms are considered the best indicators of faecal contamination [5]. In fact, even if not all coliform strains are pathogens, they primarily originate in the intestines of warm-blooded animals, hence they can be associated with faecal contamination. Nevertheless, there are many microor-ganisms (not belonging to the coliform group) commonly present in untreated water samples whose concentration largely exceeds that of coliforms. Under particular circumstances, some of these microorganisms (such as some genera of *Pseudomonas* and *Flavobacterium*) can represent a significant risk for human health.

In addition, high levels of total bacterial concentration indicate a reduction in water quality.

For these reasons, monitoring total bacterial concentration (in addition to coliforms concentration) can be a useful indi-cator of water quality.

Water microbial screening is normally carried out by Stan-dard Plate Count (SPC) method [6], which is reliable and accurate but characterized by slow response (24–72 h) and must be performed by skilled personnel in microbiology laboratories, thus further delaying the measuring time due to the need to send the samples under test to a laboratory.

A set of instruments suitable for the detection of coliforms concentration in water samples are already present on the mar-ket, as those produced by IDEXX (West brook, Maine, USA): Colilert, Colilert 18 and Colisure [7], [8]. Such systems use the detection technique named Defined Substrate Technology (DST), that is based on the coliforms property to produce β -

glucuronidase as the result of their metabolism. Water samples are introduced in 100 ml wells together with a dehydrated medium and are incubated at 37 °C for 18–22 h. At the end of the assay the number of wells whose color has turned yellow (signalling the presence of coliforms) or fluorescent yellow (because of the presence of *Escherichia coli*) is counted and the microbial concentration is inferred by statistical tables. The IDEXX instruments are laboratory oriented, their response time is only slightly shorter than SPC and color discrimination is eye based (in the case of fluorescence detection an UV lamp is required).

Research in environmental monitoring has developed many types of sensors for the screening of microorganisms [9], [10], [11] as well as for the detection of toxic pollutants in water [12], [13]. Recently, the authors have proposed an embedded system that is highly competitive with SPC in terms of measuring time (3–12 h depending on the sample contamination) and features user-friendly procedures, with no need of a laboratory environment, that allow its application for in-situ determination of bacterial concentration [14]. This system detects bacterial concentration in liquid and semi-liquid samples by using the impedance technique [15]. It works as follows. The Sample Under Test (SUT) is maintained at a constant temperature (generally in the range 30 °C–42 °C) suitable for efficient bacterial growth. At regular intervals, the SUT electrical characteristics (i.e. the impedance as well as its resistive and reactive components) are measured and plotted as function of time. After a short time needed for the electrode-electrolyte system to stabilize, measured electrical parameters remain essentially constant (baseline value) until a critical bacterial concentration (in the order of 10^7 CFU/ml) is reached. Then the SUT impedance modulus, as well as its components, begin to change significantly. Since the time needed for the electrical parameter to deviate from its baseline value, hereafter called Detect Time (DT), is linearly related to the logarithm of initial bacterial concentration, this value can easily be worked out.

The particular electrical parameter (i.e. resistive or reactive impedance component) used as monitor is essentially related to the SUT, whose chemical composition makes one impedance component more sensitive to bacterial growth than the other [16].

If the SUT doesn't contain enough nutrients to allow bacterial population to grow to the critical threshold of 10^7 CFU/ml in reasonable time (as is the case of water samples) an enriched medium must be added to the SUT. The choice of the proper medium is essential to guarantee a good trade off between bacterial growth rate and the growth medium selectivity towards a particular bacterial strain.

II. Materials and Methods

An improved version of the portable system discussed in [14] has been used to monitor bacterial concentration of water samples taken from rivers, waste waters and water-courses in the surroundings of Bologna (Italy).

A. Embedded System

The system is composed of two electronic boards: one for measuring the SUT electrical parameters (also suited for DT evaluation), while the other is a thermoregulation board controlling the SUT temperature with oscillations lower than 0.15 °C.

As discussed in [14] and [17], sample temperature must be maintained as stable as possible to avoid fluctuations of the electrical parameters that affect the measurement reliability. The SUT is placed in an incubation chamber featuring a couple of electrodes for electrical characterization and an heating system to maintain the sample to the target temperature. Two serial ports RS-232 provide the communication with an eeePC system for on-line monitoring of the assay, setting the assay parameters and data filing. Wireless data transfer can be done using a GT863-PY terminal by Telit and an Ethernet port for worldwide data transfer. A web application developed ad hoc allows data sharing with a hierarchical level of user privileges.

Fig. 1(a) presents the schematic for the different parts of the system. When receiving the start signal, the impedance measurement board enables the thermoregulation board and waits 30 minutes for the SUT temperature to stabilize. Then, it measures the SUT electrical parameters at time intervals of 5 minutes. When the monitored electrical parameter deviates from its baseline value for more than 5% the assay ends and DT is calculated according to the algorithm presented in [14].

At test signal frequencies lower than 1 MHz, the electrodes-electrolyte system can be modeled as the series of a resistance R_S, accounting for the resistance of both the sample and the electrode-electrolyte interface and a capacitance C_S (related to the formation of a double layer region at the electrode-electrolyte interface) [17].

The SUT electrical parameters have been measured using the electronic circuit (implemented in the system measurement board) represented in Fig. 1(b). A sinusoidal voltage signal V_{in} (t) (100 mV_{PP} 200 Hz) is applied to the sensor electrodes and the current drawn I_{in} (t) is measured by means of a current to voltage (I/V) converter, whose output voltage $V_{out}(t) = -(R_F Z_S) I_{in}(t)$ is linearly related with the current drawn by the sensor electrodes. Denoting with V_{Min}, V_{Mout} and φ the amplitudes of the signals V_{in} (t) and V_{out} (t) and the phase difference respectively, the SUT electrical parameters can be calculated using the aforementioned RC series model, producing the following formulas [16]:

$$K_s = (V_{Min} - V_{Mout}) R_F \cos(\phi) \quad (1)$$

$$C_s = (1 - 2\pi f R_F)(V_{Mout} - V_{Min})(1 - \sin(\phi)) \quad (2)$$

The voltage signals V_{in} (t) and V_{out} (t) are filtered (to remove high frequency and power line noise), converted into digital form and sent to ARM STR912 microcontroller for data processing.

The schematic of the thermoregulation board is presented in Fig. 1(c). The sample temperature is measured with a LM135 (National Semiconductor, Santa Clara, USA) Zener diode with a breakdown voltage proportional to the absolute tempera-ture and a slope 10 mV/°K. The voltage from LM135 is filtered and amplified by the dynamically reconfigurable Field

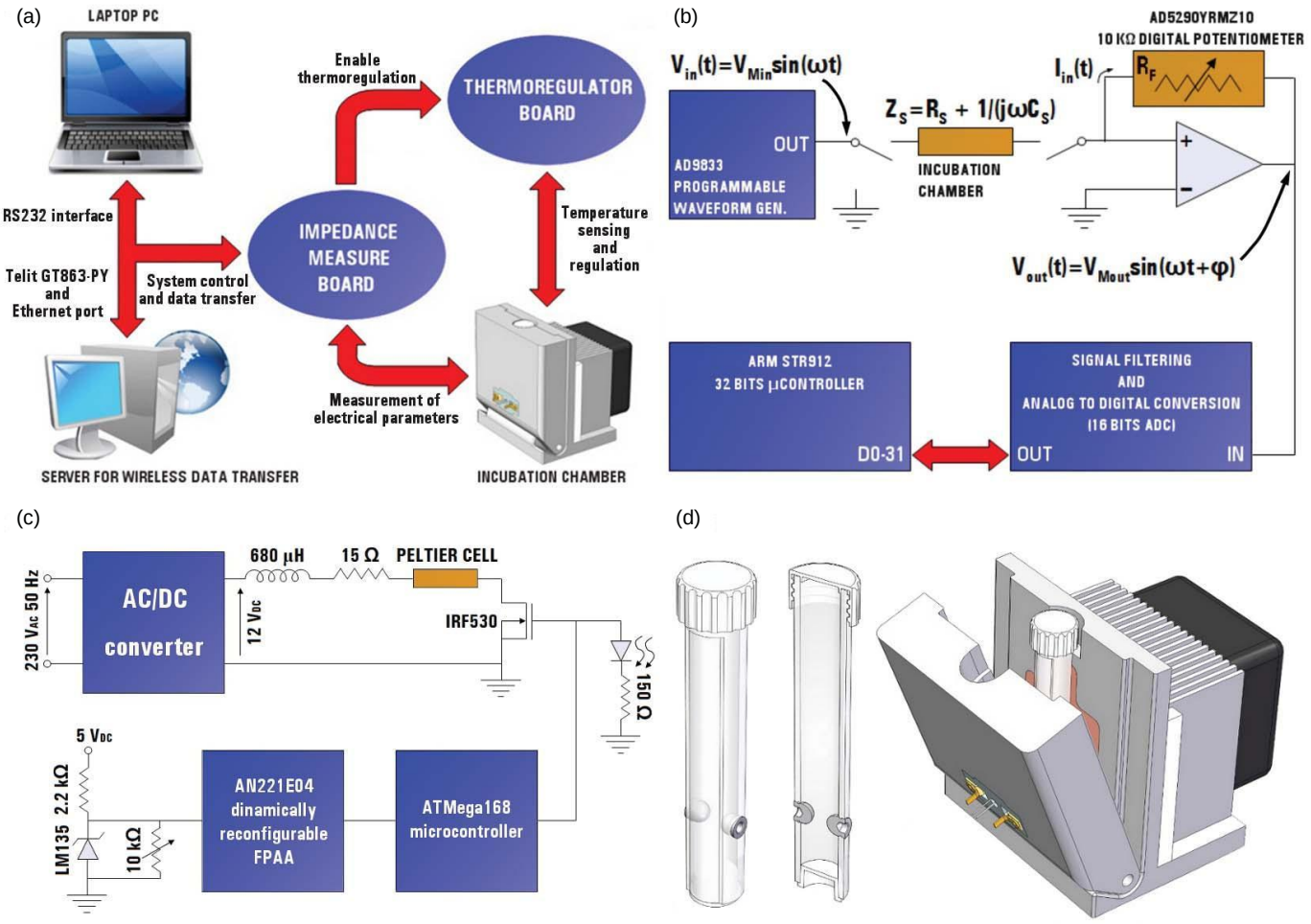

Fig. 1. (a) Schematic representation of the sensor system. (b) Circuit used to measure the sample electrical parameters. (c) Thermoregulation circuit. (d) Incubation chamber.

Programmable Analog Array (FPAA) AN221E04 (Anadigm, USA) and sent to the microcontroller ATMega168 (Atmel, California, USA) that calculates the sample temperature. The ATMega168 controls by PID algorithm the time period the n-channel power MOSFET IRF530 (Fairchild Semiconductor, USA) is turned on to supply a Peltier cell in the incubation chamber with a DC voltage of 12 V.

B. Incubation Chamber

The incubation chamber is the most critical part of the system. It must contain the sample in direct contact with the electrodes and maintain it at a constant temperature. At the beginning of each assay the chamber must be sterile so as to not contaminate the SUT and alter the assay results. Since at the end of the previous assay the sample in the chamber has reached a very high level of bacterial contamination (> 10^7 CFU/ml), the incubation chamber must undergo a sterilization process so as to eliminate the residual bacterial concentration. In the first implementation of the system [14], the sterilization process was performed by exposing the cham-ber to 100 °C steam flow for 10 minutes, a procedure that proved to be efficient for reliable measurements. Since, how-ever, steam vapor sterilization is a complicate procedure that needs dedicated instruments and is difficult to implement for in-situ measurements, recently we have developed a disposable incubation chamber allowing to avoid sterilization before use.

The new incubation chamber is composed of: a) a per-manent housing, featuring the temperature sensor, the heat-ing system and all the interconnections to the electronic boards; b) a disposable cell (Fig. 1(d)).

A representation of the different parts of the incubation chamber is shown in Fig. 2. The temperature sensor LM135 is placed in direct contact with the disposable cell to sense the SUT temperature and control the heating system accordingly. The Peltier cell (40 mm × 40 mm × 4 mm) sets the sample to the target temperature. The disposable cell, hosting the SUT during the assay, features a cylindrical structure (L 7.9 cm, W 1.3 cm) and a couple of cap shaped stainless steel electrodes (6 mm diameter, 4 mm spaced) that are connected to the housing by means of sliding contacts. The temperature of the disposable cell is due to thermal exchange with the housing. Before starting the assay, a new (sterile) sensor cell is filled with the SUT and it is inserted in the housing. At the end of the assay it is extracted and be disposed of.

C. Chemicals and Media

The study has been carried out on real water samples taken from rivers, wastewaters and watercourses in the surrounding

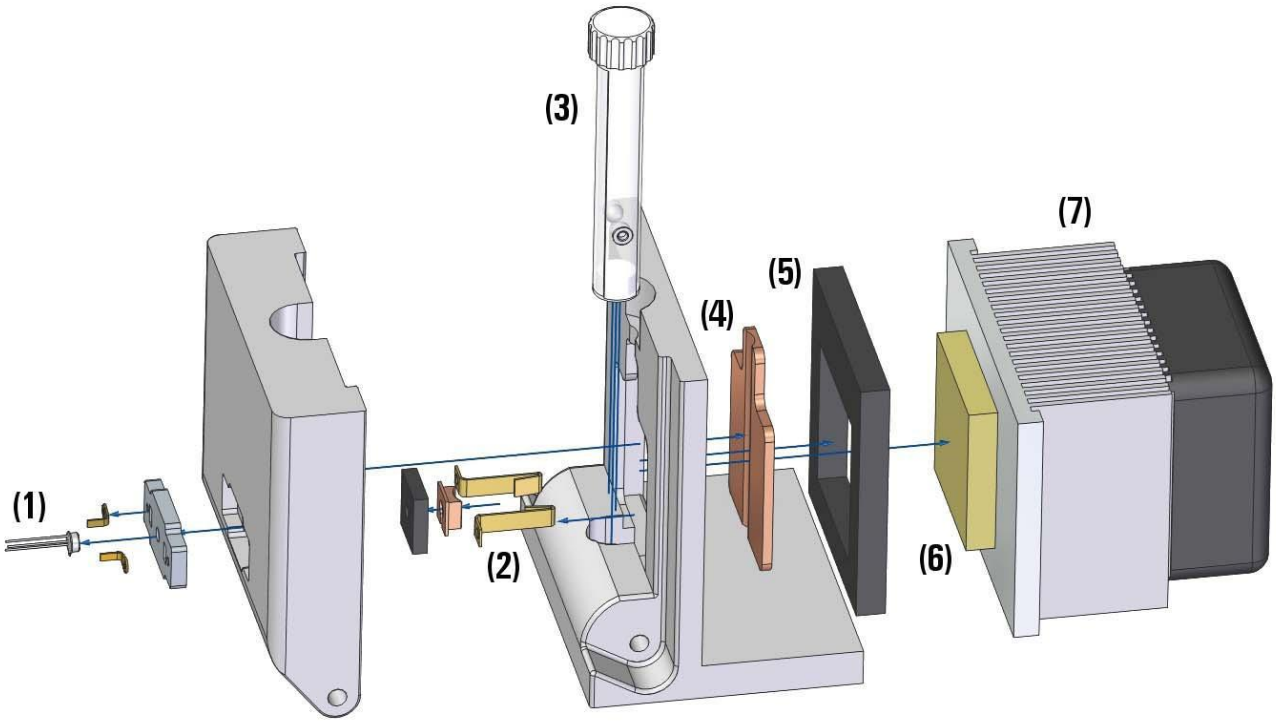

Fig. 2. Representation of the different parts of the incubation chamber. (1) LM135 temperature sensor. (2) Sliding contacts for the disposable cell electrodes. (3) Disposable cell. (4) Thermal spreader. (5) Thermal insulation. (6) Peltier cell. (7) Fan-operated heat sink.

of Bologna (Italy) and stored at 4 °C for 24–48 h before use.

The tested samples have been diluted in enriched medium (ratio 1 : 10) to provide nutrients for bacterial growth. Three different enriched media have been tested: Lauria Bertani (modified to feature low salt concentration) for total bacterial concentration; Mc Conkey Broth and Lac-tose Broth for coliforms concentration. The composition (for 1 liter of distilled water) for the three media is as fol-lows. Lauria Bertani: Tryptone 10.0 g, Yeast Extract 5.0 g (pH 7.0). Mc Conkey Broth: Oxgall 5.0 g, Peptone 20.0 g, Lactose 10.0 g, Bromcresol Purple 0.01 g (pH 7.3). Lactose Broth: Beef Extract 3.0 g, Peptone 5.0 g, Lactose 5.0 g (pH 6.9).

All the ingredients are purchased from Difco Laboratories (Detroit, USA). The incubation temperature has been set to 37 °C.

SPC measures of microbial concentration have been carried out immediately before the assay to test the correlation with the data obtained with the system of this work. Lauria Bertani agar has been used for total bacterial concentration and Mc Conkey agar for coliforms concentration.

D. Statistical Analisys

Statistical analysis has been carried out on the experimental data using Microsoft Excel. Linear regression analysis has been used to estimate the relationship between measured DTs and logarithm of bacterial concentration determined by SPC. The regression line equation as well as the determination coefficient R^2 have been calculated and the kinetics growth parameters for the bacterial population have been determined from the regression line equation.

III. Results and Discussion

Water samples have been tested using the system of this work and the results have been compared to the microbial concentrations measured by SPC.

The monitored curves for R_S and C_S (resistive and capacitive components of the impedance Z_S , respectively) are shown in Fig. 3 vs. total microbial concentration in the case of samples diluted in Lauria Bertani medium in ratio 1:10.

As can be seen, lower contaminated samples are characterized by higher values of measured DT, while samples with high bacterial concentration feature low values for DT. With reference to Fig. 3, a bacterial concentration of 300 CFU/ml needs more than 10 h to be detected while in the case of a strongly contaminated sample ($3.5 \cdot 10^6$ CFU/ml) it takes less than 3 h.

The choice of the enriched medium to dilute the water sam-ple significantly affects the system performance (response time and accuracy), since the system detects the electrical changes in the medium due to bacterial metabolism. In general, large percent variations of the monitored parameter is desirable since this results in more reliable and accurate DT determination. Fig. 4 shows the percent change of R_S and C_S for all three enriched media (error bars indicate the dispersion, i.e. standard deviation): as can be seen the resistive component produces slightly larger variations than C_S for all media but Mc Conkey medium, characterized by limited percent variations in R_S due to the high bile salt concentration (Oxgall), partially masking

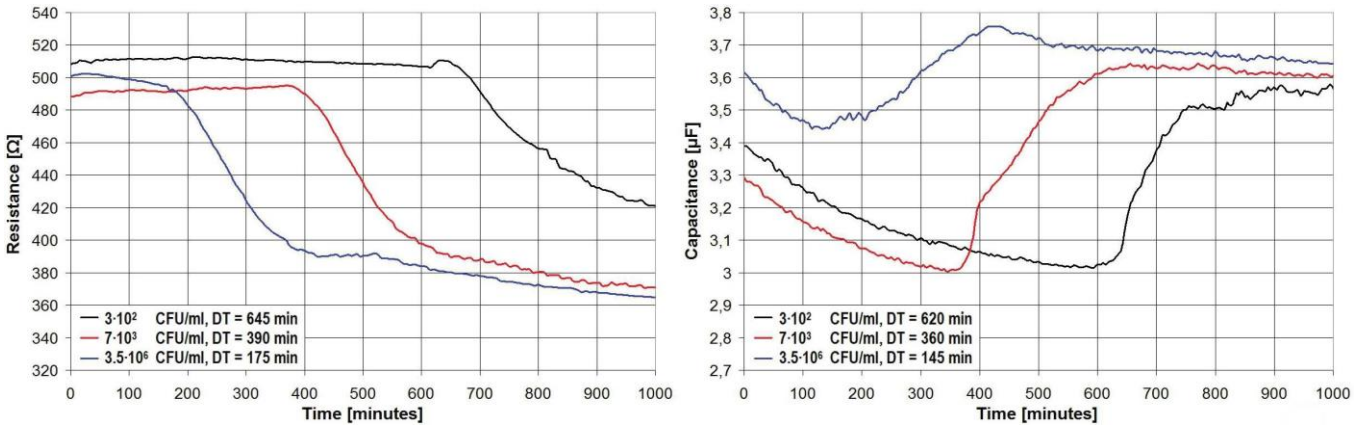

Fig. 3. Resistive and capacitive components of the impedance Z_S versus time for water samples characterized by different amounts of total bacterial contamination.

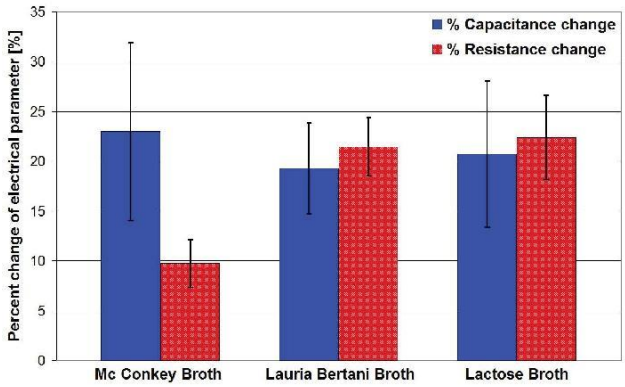

Fig. 4. Percent change of R_S and C_S , due to bacterial metabolism, for the three enriched media that are used to dilute the water sample.

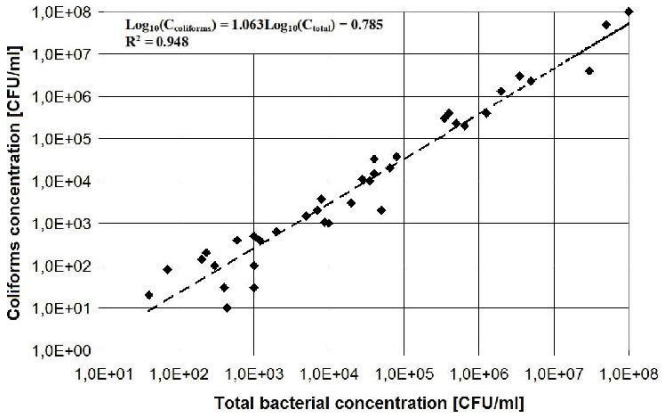

Fig. 5. Scatter plot of coliforms concentration versus total bacterial concentration (measured by SPC).

the increased ion concentration by bacterial metabolism. The variation of R_S , instead, exhibits much lower dispersion than that of C_S . This can be related with the different physical causing the variation of the SUT electrical parameters. As bacterial population grows, microbial metabolism transforms uncharged particles in the medium in highly charged ones, thus modifying the ionic content of the SUT and increasing its bulk conductivity (hence R_S decreases). The ions in the electrolyte are subjected to different electrical forces at the electrodes and in the bulk of the SUT, thus leading to the formation of a double layer region at the electrode-electrolyte interface (hence C_S increases). This interface capacitance dominates the total impedance value at low frequency.

Fig. 5 shows coliforms bacterial concentration (measured by SPC in Mc Conkey agar) vs. total bacterial concentration (measured by SPC in Lauria Bertani agar) for the entire set of water samples that have been tested. A linear relation exists between the logarithm of the two concentrations as pointed out in the figure inset, presenting both the linear regression line equation and the determination coefficient R^2. The col-iforms concentration in tested samples is about one order of magnitude lower than the total microbial concentration with good linearity between the two variables as indicated by the high value of the determination coefficient (R^2 = 0.948).

Water samples have been tested for all three enriched media and both electrical parameters. Fig. 6(a)–(c) show scatter plots (representing the measured DT vs. the logarithm of bacterial concentration determined by SPC) for the three enriched media in the case of DT calculated from R_s plots (scatter plots from C_s results in slightly higher dispersion, i.e. lower correla-tion between DT and bacterial concentration). In each figure the linear regression line equation, determination coefficient R^2 as well as lower and higher bounds resulting from a Student t-distribution statistic with a confidence level of 95% are presented. Lauria Bertani medium and Lactose Broth resulted in comparable accuracy with SPC (with values of R^2 of 0.772 and 0.766 respectively) while the use of Mc Conkey medium produced lower correlation (R^2 = 0.609).
Based on the linear regression line equation, the esti-mated value of bacterial concentration C_0 has been calcu-lated. Since the statistical distribution for the random variable

$Log_{10}\, C_0\, C_{S\,PC}$ is known to be Gaussian [17], this has been assumed to calculate the distribution mean value and standard deviation. Fig. 6 (d) shows probability density functions of the random variable $Log_{10}\, C_0\, C_{S\,PC}$. As can be seen Lauria Bertani medium and Lactose Broth are characterized by almost

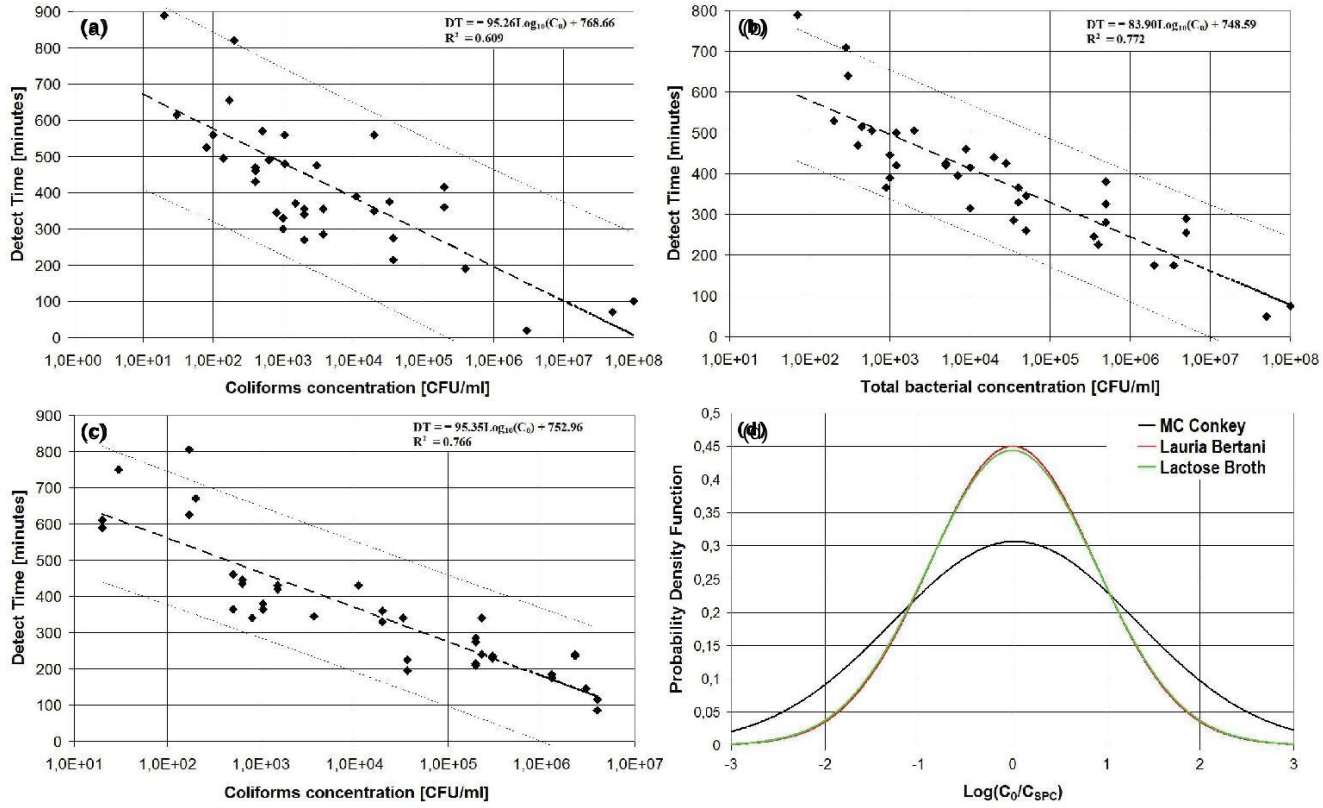

Fig. 6. Scatter plots of measured DT versus microbial concentration for the three enriched media. (a) Mc Conkey Broth. (b) Lauria Bertani. (c) Lactose Broth. (d) Probability density function for the Gaussian variable for the three enriched media.

TABLE I

ESTIMATED VALUES OF T_G , T_{lag} , AND R_2 FOR THE THREE ENRICHED MEDIA. RESULTS OBTAINED THROUGH MONITORING THE RESISTIVE AND CAPACITIVE COMPONENTS OF THE IMPEDANCE ARE SHOWN

	Parameters Measured From the Resistance Curves			Parameters Measured From the Capacitance Curves		
Enriched Medium	T_G (min)	T_{lag} (min)	R^2	T_G (min)	T_{lag} (min)	R^2
Mc Conkey Broth	28.78±7.73	103.27±95.77	0.609	26.64±6.87	73.83±85.11	0.614
Lauria Bertani	25.18±4.76	160.96±48.41	0.772	26.18±5.18	122.23±52.68	0.750
Lactose Broth	28.39±5.53	89.59±61.73	0.766	29.33±6.05	75.80±67.64	0.728

identical distributions while the Mc Conkey broth distribution (featuring significant higher value of standard deviation) results in wider dispersion for the estimated bacterial concentration.

Repeatability tests have been carried out on a limited num-ber of samples to study the uncertainty in DT determination. The same sample has been tested with three different assays and the DT standard deviation σ recorded. The results show that for Lauria Bertani medium σ is 10.2 minutes, for Lactose Broth is 7.06 minutes while, in the case of Mc Conkey broth, is 32.9 minutes. As a comparison, the corresponding standard deviation for the whole set of water samples is 113 minutes for Mc Conkey broth, 42.98 minutes for Lauria Bertani medium and 46.63 minutes for Lactose Broth. This suggests that dispersion in scatter plots of Fig. 6 is mainly due to differences in the growth speed of the different bacterial strains.

The scatter plot data of Fig. 6 can be used to analyze the dynamics of bacterial growth. As pointed out in [17], one can assume the linear regression equation $DT = A \times Log_{10}(C_0) + B$, where C_0 is the initial unknown bacterial concen-tration, and denote: T_G the mean generation time (i.e. mean time between cell duplication); T_{lag} the lag time needed for the microbial strains to adapt themselves to growth conditions; C_T the critical threshold concentration of 10^7 CFU/ml and τ the 30 minutes time delay for the sample temperature to stabilize. Then, it is:

$$C_T = C_0 \times 2^{(DT + \tau - T_{lag})/T_G} \quad (3)$$

Comparing Eq. (3) with the linear regression equation it is:

$$T_G = -A \times Log_{10}(2) \quad (4)$$

$$T_{lag} = B + \tau + A \times Log_{10}(C_T) \quad (5)$$

Statistical analysis has been carried out to test if any significant difference exist for the regression line parameters in the cases of the three enriched media (with a confidence level of 95%). The obtained results indicate that no significant

differences exist in the intercept B of the regression lines for the different enriched media, while media used for selective coliforms detection (Mc Conkey and Lactose Broth) are caracterized by significantly higher values of the slope A than in the case of Lauria Bertani.

Table I presents the estimated values for T_G , T_{lag} and R^2 for the three enriched media as obtained with both the resistive and capacitive component of the impedance.

IV. Conclusion

An embedded portable sensor system featuring a disposable measurement cell has been presented. The system is particularly suitable for in-situ detection of bacterial concentration in water samples and is competitive with the standard technique in terms of time response (3–12 h vs. 24–72 h) and possibility to be implemented in automatic form.

The system has been used to test the microbial concentration in water samples from different sources (rivers, wastewaters, watercourses), that have been diluted in a suitable enriched medium. Three different media have been tested and the system response has shown good correlation with the stan-dard technique (in particular for the enriched media Lactose Broth and Lauria Bertani). By using the appropriate enriched medium either coliforms or total bacterial concentration can be reliably estimated with response time as low as 3 h for highly contaminated samples (> 10^6 CFU/ml). Furthermore, diluting the water samples in specific enriched medium the determination can be made selective for different types of bacteria.

On the whole, the obtained result indicates that the presented system is a reliable tool for fast and in-situ water monitoring.

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