Study toward the Integration of a System for Bacterial Growth Monitoring in an Automated Specimen Processing Platform

Paolo Bellitti, Michele Bona, Stefania Fontana, Emilio Sardini, and Mauro Serpelloni

Abstract. As bacterial infection diseases represent a relevant threat for human health worldwide, many efforts are spent in accelerating the diagnostic process of biological specimens. The WASPLab automated platform, by COPAN Italia S.p.A., detects bacterial growth by processing the images of the Petri dishes containing a sample to analyze. This work presents a study performed on a developed system that exploits impedance measurement to monitor bacterial growth in Petri dishes in real time. It is part of an activity aiming at system integration in the WASPLab, to enhance its monitoring capabilities and flexibility. Through repeated 24-hours tests executed with the system, we successfully detected *S. aureus* growth in Petri dishes that were inside one of the WASPLab incubators, starting from impedance measurements performed at 50 Hz and 150 Hz. In particular, depending on the parameter being observed, detection time was between four and six hours, for an initial bacterial concentration in the order of $4.5 \cdot 10^7$ CFU/ml. These preliminary results represent the first step for evaluating system integration in the WASPLab.

Keywords: Bacterial Growth Detection, Impedance Measurement, WASPLab Platform.

1 Introduction

Bacterial infections count as a major source of disease worldwide, especially when they are not properly treated. As an example, nearly 50 million infection-related sepsis cases are estimated each year, with more than 5 million deaths [1]. The problem is even more serious because of the increasing capability of some bacterial strains to resist to specific antibiotic therapies, as indicated in a report released in January 2018 by the World Health Organization [2]. For these reasons, a quick diagnosis showing that a biological specimen is infected needs to be achieved, since it allows delivering a proper therapy in a timely manner, preserving subject's health.

The market provides numerous commercial systems able to meet this necessity. In particular, such systems detect bacterial growth in a biological sample much faster

than traditional methods based on colony count [3-4]. In this way, only the specimens that are really contaminated are sent sooner to dedicated laboratories for specific tests, accelerating analysis process and therapy delivery to a patient. They exploit different measurement principles for their operation. For instance, the BacT/ALERT (bioMérieux SA) [5-6] and the BACTEC™ (BD) [7] detect a fluorescence variation, which is due to CO_2 production from bacterial metabolism. The BacTrac (SY-LAB Geräte Gmbh) [8] and the RABIT (Don Whitley Scientific Ltd.) [9] perform a measurement of electric impedance, which changes because of pathogens activity. Then, the Biacore (GE Healthcare) exploits the principle of Surface Plasmon Resonance to detect the presence of bacteria on sensor surface [10]. Finally, the Thermal Activity Monitor (TA Instruments) evaluates bacterial growth from the heat generated by related chemical reactions, implementing isothermal microcalorimetry method [11].

An approach different from those followed by the previously mentioned systems is exploited by the WASPLab platform (COPAN Italia S.p.A.) [12]. WASP stays for Walk-Away Specimen Processor. Referring to Error! Reference source not found., which shows it for convenience, the WASPLab works in the following way. A Petri dish is inoculated with a possibly infected biological sample in WASP station, by a robotic manipulator. Then, it moves to one of two temperature-controlled incubators, in order to enhance bacterial growth, which is monitored by taking pictures of it at different moments in the image acquisition stations. Pictures are analyzed from a digital imaging interface, and they are stored in WASPLab Central, which shares data about the analysis to other connected systems. Finally, dishes are accumulated in silos at the end of the analysis process. All steps are managed in a completely automated way.

Fig. 1. Picture of the WASPLab automated specimen processing platform.

The WASPLab platform is a very advanced solution for bacterial growth detection. However, there is the possibility to even enhance its capabilities and flexibility. For instance, analyzed Petri dishes need to be moved between incubators and image ac-

quisition stations every time a picture has to be taken. This repeated change in the environmental conditions may have a negative influence on bacterial growth. Then, pictures of two Petri dishes at most can be acquired at a time, because there are two image acquisition stations in the WASPLab, each receiving the Petri dishes from one of the incubators. Finally, carrying out a measurement with a sensor system would permit to have at disposal quantitative data about bacterial growth in real time.

In a previous work [13], we described a system that performs this task through repeated impedance measurements related to a Petri dish. Such system has been developed with the final aim of integrating it in the WASPLab once it is optimized, in order to monitor bacterial growth in Petri dishes while they remain always inside the incubators. Furthermore, we illustrated an experimental analysis executed in a laboratory setup, to evaluate system performances regarding measurement accuracy and bacterial growth monitoring. On the other hand, this work presents a study performed with the system operating when analyzed Petri dishes were incubated directly inside the WASPLab. Through the study, we could observe system behavior in the field, which is the first step toward its integration in the WASPLab. The following section includes a brief description of the system and illustrates how the study was carried out. Then, third section presents the obtained preliminary results.

2 Materials and Methods

2.1 The Measuring System

For this work, we realized an improved version of the measuring system, with respect to the one presented in [13], which enhances its portability. Used system is shown in Error! Reference source not found.. It presents three different principal parts.

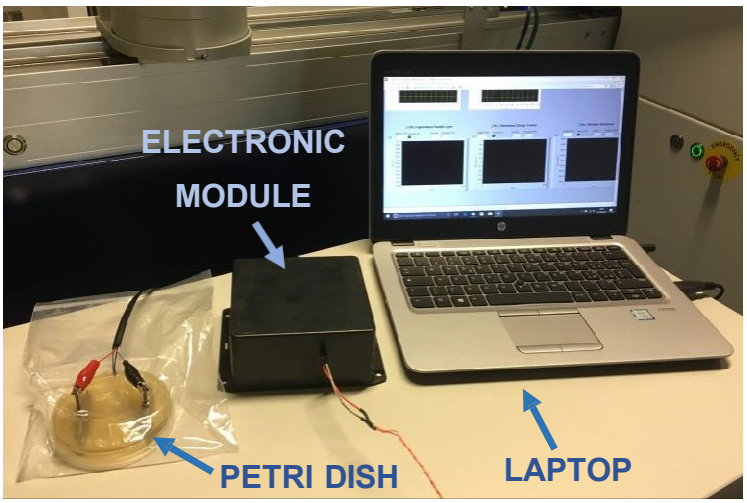


Fig. 2. The measuring system in its improved version.

The first part is an instrumented Petri dish, which has been realized by adding two macroelectrodes of specific geometrical characteristics to the same disposable object that is used during the analysis with the WASPLab. It contains an agar-based culture medium for bacterial proper growth. From the frequency response of the instrumented Petri dish, we obtained an equivalent lumped-parameter model representing either electrode/medium interface, through double layer capacitance C_{DL} and charge transfer resistance R_{CT}, or medium conductivity, through resistance R_M. These parameters are the quantitative data about bacterial growth that the developed system is able to provide. They are obtained from Petri dish impedance measurements performed at two fixed working frequencies, by implementing specific mathematical formulas [13].

The second part is an electronic module, which is designed for measuring the impedance related to the instrumented Petri dish at the working frequencies [13]. It is entirely inside a box of dimensions 120 x 120 x 55 mm. Then, it is configured according to a particular necessity, by properly choosing its components. The system can work with more electronic modules, if the number of the Petri dishes to analyze simultaneously augments. This increases its modularity.

Then, the third part is a laptop, executing a LabVIEW Virtual Instrument (VI) that drives the electronic module and elaborates the impedance measurements to find C_{DL}, R_{CT}, and R_M. This VI allows system user changing measurement parameters when necessary and monitoring bacterial growth by looking at C_{DL}, R_{CT}, and R_M real time trend. Furthermore, it presents additional features, with respect to the program described in [13]. First, since the system is designed to implement an automatic bacterial growth monitoring, it generates an alarm as it detects that C_{DL}, R_{CT}, or R_M variations have overcome a threshold. Second, when the alarm occurs, an e-mail is sent automatically to a defined address, reporting that bacterial growth has been detected and at what time. Third, obtained data are stored not only in laptop's own memory, but also in a folder that is shared with connected devices through the cloud. In this way, information about bacterial growth is available from remote locations too.

2.2 Study setup and protocol

The study presented in this paper was entirely carried out in COPAN Italia S.p.A. (Brescia, Italy), with the developed system working with the WASPLab platform, as shown in Error! Reference source not found.. During the study, we executed repeated bacterial growth monitoring tests with the same setup, whose block scheme is represented in Error! Reference source not found.. Furthermore, every test was conducted following a defined protocol.

Three instrumented Petri dishes were filled with Tryptone Soy Agar medium. Two of them were also inoculated with *S. aureus* ATCC 6538, considering an initial concentration in the order of $4.5 \cdot 10^7$ CFU/ml. Such value is high, but we kept it to verify that the system worked properly, in a favorable condition. Inoculation took place in a safe environment, at ambient temperature. Then, remaining Petri dish was not inoculated. In this way, we had a reference to evaluate the trend related to the others.

The next step after inoculation was system preparation. We configured three electronic modules, in a way they excited one Petri dish each with waveforms of amplitude equal to 1 V_{pp} and measured Petri dish impedance at frequencies f_1 = 50 Hz and

Fig. 3. System working with the WASPLab platform.

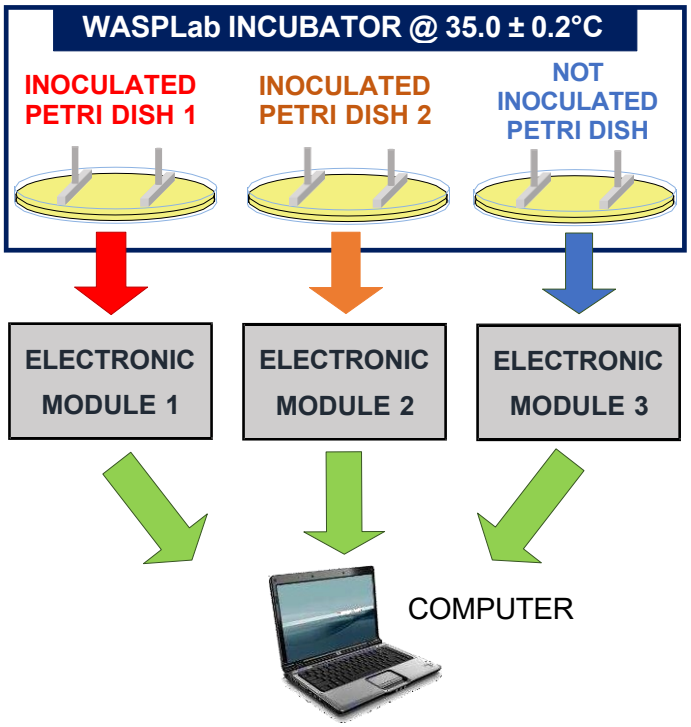


Fig. 4. Block scheme of the setup used for testing the system during the study.

f_2 = 150 Hz. In addition, their internal gain factor was obtained by attaching a 100 Ω commercial resistor to the system and acquiring the corresponding impedance.

When the system was ready, we put the Petri dishes in one of the WASPLab incubators, which had already turned on, in order to make its internal temperature uniform. In particular, once incubator was closed after Petri dishes positioning, its control system drove the temperature to stay in a range between 34.8 °C and 35.2 °C, given a set point equal to 35.0°C. Every Petri dish was attached to the terminals of an electronic module. As all modules are equal to each other, such operation was performed without respecting a precise order.

Last step was triggering the VI running on the laptop to acquire bacterial growth data. We set its parameters to allow a continuous impedance measurement at f_1 and f_2 and provide a single value of C_{DL}, R_{CT}, and R_M every two minutes, for 24 hours.

3 Preliminary results

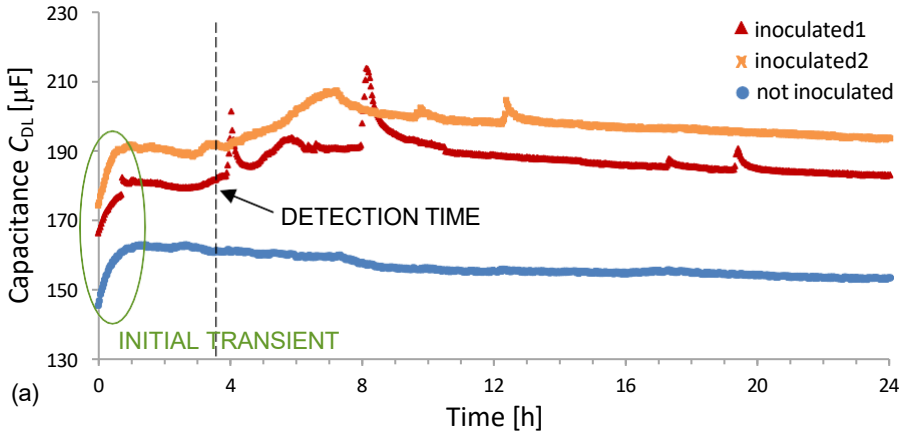
3.1 Double layer capacitance C_{DL}

Error! Reference source not found. illustrates C_{DL} time behavior related to the Petri dishes analyzed during one of the tests. All represented curves have an initial transient

of about one hour, which is due to medium temperature variation from initial state (inoculation at ambient temperature) to the condition in which dishes are inside WASPLab incubator (about 35 °C). Then, once initial transient gets over, the curve related to the Petri dish that was not inoculated presents a gradual decreasing trend, caused by medium partial drying. On the contrary, the curves referring to inoculated Petri dishes reflect a typical growth trend. In fact, after a lag phase in which it is stable, C_{DL} starts to augment (log phase). Such increase derives from bacterial growth, which generates charged molecules accumulating at electrode/medium interface, as reported in the literature [3]. The transition between lag phase and log phase identifies system detection time, which occurs at four hours. Finally, the curves show a brief stationary phase, in which C_{DL} is stable again, and death phase, in which C_{DL} decreases, as bacterial activity is coming to an end. Even though both curves allow recognizing all growth phases, they are characterized by a different dynamics. For instance, log phase lasts about one hour less for inoculated Petri dish 1 than for inoculated Petri dish 2. This is caused by a variance in bacterial distribution along electrode/medium interface between the two inoculated Petri dishes, as inoculation step was carried out by hand and, therefore, it is not an exactly repeatable operation. In fact, a low variability in double layer characteristics may have great consequences on C_{DL} values. In any case, such discrepancy does not lead to a relevant difference in detection time.

3.2 Charge transfer resistance R_{CT}

Error! Reference source not found. shows the time trend characterizing the other interface parameter, i.e., resistance R_{CT}. This figure highlights that R_{CT} behavior reflects C_{DL} curves shape shown in Error! Reference source not found.. In fact, all curves present an initial transient and those related to inoculated Petri dishes help identifying bacterial growth phases, even if with different dynamics.



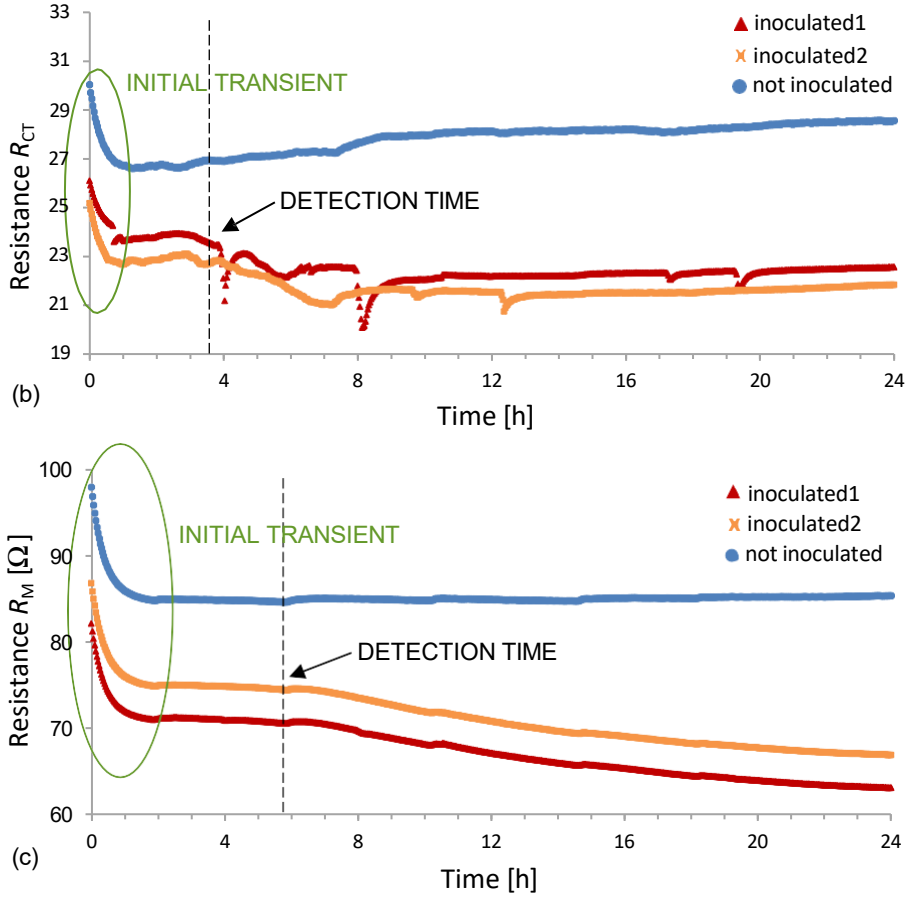


Fig. 5. Time trends of parameters providing information about bacterial growth, obtained from one of the performed tests. (a) Capacitance C_{DL}. (b) Resistance R_{CT}. (c) Resistance R_M.

However, R_{CT} has an opposite direction with respect to C_{DL}. In fact, for every C_{DL} increasing tract, there is a R_{CT} descending phase, and vice versa. In particular, bacterial growth leads to a decrease of R_{CT}, since it causes an accumulation of charged molecules at electrode/medium interface, which augments the charge transfer capability of double layer. Since R_{CT} and C_{DL} trends are similar, system detection time extracted from the analysis on charge transfer resistance is comparable to the one obtained from observing double layer capacitance.

3.3 Medium resistance R_M

Finally, Error! Reference source not found. represents R_M time behavior, which is identical for the two curves related to the inoculated Petri dishes (although there is a difference between the corresponding values), unlike what happens for C_{DL} and R_{CT}.

Furthermore, Error! Reference source not found. highlights that such behavior does not reflect the one characterizing the other parameters. In fact, after a two-hours initial transient, R_M decrease during log phase for inoculated Petri dishes is slower than R_{CT}'s. In addition, this trend lasts until the end of the test. Anyway, decrease is in agreement with what is stated in the literature, as it is due to an increase of medium conductivity caused by bacterial metabolism [3]. Consequently, system detection time obtained from R_M observation is about six hours, i.e., it is greater than what is found from C_{DL} and R_{CT} study. On the other side, the curve related to not inoculated Petri dish has a progressively increasing trend after the transient, which is caused by medium partial drying.

Even though its analysis leads to the identification of a greater detection time, as compared to those found by studying C_{DL} and R_{CT} trends, R_M has a more stable behavior. In fact, its curves do not present the anomalous peaks that are visible when observing the other two parameters. Consequently, since the measuring system has the advantage of monitoring three parameters at the same time, a preliminary alert about bacterial growth detection can be generated from C_{DL} and R_{CT} observation. Then, a full alarm can be produced when the system detects R_M variation too.

4 Conclusions

This paper has presented a study performed on a system designed for bacterial growth monitoring in Petri dishes. System operation relies on the impedance measurement, at two fixed working frequencies, of the analyzed Petri dishes, which are instrumented with electrodes. Firstly, we have given a general description of the system. Then, we have illustrated how the study was conducted, i.e., *S. aureus* growth was monitored when Petri dishes were inside an incubator of an automated platform, called WASPLab and commercialized by company COPAN Italia S.p.A. Finally, we have reported preliminary achieved results, which highlight system capability to detect bacterial growth in the field.

Preliminary results from the performed study pave the way for system integration in the WASPLab platform or, at least, for a connection between them, once the former is optimized for such purposes. This would lead to several advantages regarding the automated analysis carried out by the WASPLab. First, bacterial growth is monitored in Petri dishes that are inside the incubators for the entire test duration. Second, since the system provides three output parameters, additional real time data about any growth phase is obtained, from all analyzed Petri dishes. Such data could be shared between other connected systems, included the remote ones, allowing all authorized people to be informed anytime and anywhere. Finally, the system is easily reconfigurable, to meet user's needs and particular applications. This contributes to augment WASPLab flexibility.

Future research activity will deal with further growth tests, considering different initial concentrations and different bacteria. In addition, design features for system optimization will be introduced, in order to favor its integration in the WASPLab.

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