Study for the integration of a measuring system to an automated platform for monitoring the growth of bacterial cultures

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***Abstract*—As bacterial infections are still a risk for human health, the market offers different systems able to detect bacterial growth in biological samples. One of them is the WASPLab automated platform, commercialized by COPAN Italia S.p.A., which combines growth detection capabilities with high levels of automation and connectivity, in compliance with Industry 4.0 principles. In this paper, we describe a study carried out on a system, whose operation relies on impedance measurements, to evaluate the possibility of its integration to the WASPLab. This integration would provide a larger quantity of data about bacterial growth to the user and would optimize analysis process. By using this system, we observed the growth of *S. aureus* in Petri dishes, while they were directly inside one of the WASPLab incubators. System provided enough information to successfully detect bacterial growth with detection time equal to three, four and a half, and six hours when initial pathogen concentration was in the order of $4.5 \cdot 10^8$ CFU/ml, $4.5 \cdot 10^7$ CFU/ml, and $4.5 \cdot 10^6$ CFU/ml, respectively. Results highlight that the measuring system could work together with the WASPLab, enhancing its monitoring performances, connectivity, and flexibility. This contributes to the realization of an architecture compliant with the model of the Factory of the Future.**

Keywords— bacterial growth monitoring; detection time; impedance measuring system; WASPLab automated platform.

I. INTRODUCTION

Bacterial infections are still widespread all over the world, with no exceptions. For instance, it is estimated that 52% of African population could potentially be in contact with contaminated drinking water. In addition, such percentage is as high as 14% in Europe [1]. If not properly treated, infections can lead to severe diseases that put human health at risk. The situation is complicated by the fact that a number of bacterial strains are able to resist to antibiotic therapies, as illustrated by World Health Organization's last report [2]. Consequently, the early detection of the presence of an infection in a biological sample is fundamental to save patient's health.

In the last decades, the market has continuously offered systems able to detect bacterial growth more rapidly than through conventional count methods [3]–[5]. In this way, at least a discrimination between contaminated and not contaminated samples was possible, before sending them to specialized laboratories for complex and time-consuming tests. Such systems work based on different principles [3]–[8]. Some examples are provided in [9]–[14].

Among these examples, the WASPLab (Walk-Away Specimen Processor) platform [14], commercialized by COPAN Italia S.p.A., operates through a specific approach. A Petri dish is inoculated with a potentially infected sample and it moves into an incubator. Then, at specific time intervals, it goes to an image acquisition station and comes back to the incubator. In this way, the growth of bacterial cultures is observed thanks to the elaboration of Petri dish images. Furthermore, the WASPLab has features that combine growth detection capabilities with high levels of automation and connectivity with other smart systems, in compliance with Industry 4.0 principles [15]. First, Petri dishes are inoculated by a robotic manipulator and moved between incubator and image acquisition station in a completely automated way. Second, environmental conditions inside the incubator (e.g., temperature and air flow) can be controlled from a decentralized station, integrated to the incubator itself, to assure a proper bacterial growth. Then, images are elaborated by a processing software and are visualized through a user interface. Finally, a station called WASPLab Central stores all images in the cloud and creates a communication through which data about bacterial growth can be shared between the platform and collaborating systems. All these features make the WASPLab a player in the world of "Digital Bacteriology".

However, especially in the Industry 4.0 era, any system should always renew itself to quickly respond to the necessities of a world that is continuously changing (e.g., evolving market needs, increasing requirements for product customization, etc.) and increase its throughput. If we compare the WASPLab with a smart manufacturing machine and bacterial growth monitoring with a production process, we can identify some disadvantages in the analysis carried out by this platform. First, Petri dishes iterative movement between incubator and image acquisition station exposes them to a frequent change of internal environmental conditions, which could affect bacterial activity. Secondly, images of only two Petri dishes can be

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The present research activity is part of the Adaptive Manufacturing project (CTN01_00163_216730), which is financed by the Italian Ministry for the Instruction, University, and Research, through the Italian "Cluster Tecnologico Nazionale Fabbrica Intelligente".

taken at a time, since the platform is equipped with two image acquisition stations, each coupled with an incubator.

In this framework, we identify metrology as an enabling instrument to optimize WASPLab analysis process. In particular, in a previous work [16], we presented a flexible and modular system able to provide quantitative data about bacterial growth from Petri dish impedance measurement. In addition, we described the laboratory tests carried out in order to assess system capabilities in terms of measurement accuracy and growth detection. On the other side, this paper illustrates a study, during which we tested the system in the field, i.e., while analyzed dishes were directly inside one of the WASPLab incubators. In this way, we could evaluate the possibility of its integration to the WASPLab. Paper following section provides some information about the developed system. Then, third section describes the performed study, presenting either test protocol or achieved results.

II. THE MEASURING SYSTEM

A. Description

Fig. 1 shows a picture of the measuring system, which is able to work autonomously. Although its parts have been already described in detail in [16], this section gives a brief description of it, for convenience. It is composed by three main parts, which are highlighted in Fig. 1.

The first part is a disposable Petri dish, containing a medium that assures a proper growth of bacterial cultures that are inoculated inside. It is similar to those analyzed by the WASPLab. The only difference is that it is instrumented with an electrode-based sensor. In particular, sensor is made by a couple of steel macroelectrodes, whose geometric configuration was designed in a way to measure Petri dish impedance covering the greatest area as possible. Furthermore, we fitted system frequency response with an equivalent model, whose parameters are double layer capacitance C_{DL} and charge transfer resistance R_{CT}, which describe the behavior at electrode/medium interface inside the Petri dish, and medium resistance R_{M}. Such parameters can be calculated through mathematical equations, starting from impedance measurement at two frequencies [16]. They represent system final outputs, and provide the required information about bacterial growth.

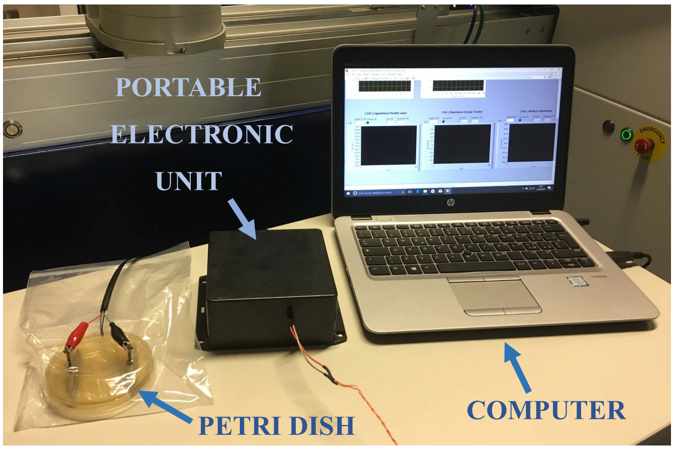


Fig. 1. The developed measuring system.

The second part is a portable electronic unit, which excites the sensor in the Petri dish with established sinusoidal waveforms and evaluates its impedance response. It is mainly composed by AD5933 integrated circuit (Analog Devices, Inc.), a microcontroller, an analog front-end, and a clock source represented by an oscillator with a phase-locked loop divider. Clock frequency can be adjusted according to the measurement frequency range, by acting on the source. Alike, analog front-end can be adapted to the attached load (i.e., the instrumented Petri dish). This allows unit reconfiguration if it becomes necessary for a specific application. The unit is contained in a box, which has dimensions equal to 120 x 120 x 55 mm, favoring its portability. In addition, it can be replicated if the number of Petri dishes to analyze increases, augmenting system modularity.

Finally, the third part is a computer, which supplies the electronic unit from its USB port and runs an interface program managing unit operation. In particular, it permits to set the values of different measurement parameters, such as working frequencies and test duration, according to the specific application. This gives a good level of flexibility to the system too. Then, the program acquires the impedance measurements and elaborates them to find the values of parameters C_{DL}, R_{CT}, or R_{M}. Furthermore, it presents them through graphs and numerical indicators, in a way that the user can monitor bacterial growth in real time. Finally, the computer stores all the obtained data.

As compared to the version illustrated in [16], we added some functionalities to the interface program, which enhance system connectivity with other devices and favor its operation in the field. First, once C_{DL}, R_{CT}, or R_{M} vary enough with respect to a proper threshold defined by the user, the program produces an alarm reporting bacterial growth detection. In particular, a pre-alert becomes active if at least one of these parameters overcomes the threshold. Then, there is a full alert when this happens for all parameters. In addition, every time such alarms are generated, an e-mail is automatically sent to a specified address, exploiting SMTP server, since computer is connected to a dedicated network (such as the company network). The message contains which parameters changed and at what time after test beginning (i.e., growth detection time). The second functionality regards data storage. In fact, they are saved in a folder of the computer, which is shared via cloud with connected systems. Third, access to computer desktop from allowed remote devices is possible at any time of the test in course. All implemented functionalities permit to system user to monitor its operation in real time and to be informed that bacterial growth occurred, even if he/she is not in the same location as the one where analysis process is taking place.

B. Operation scenarios

Fig. 2 represents a schematic of a scenario involving the measuring system working with the WASPLab platform. In the figure, only the interested WASPLab components are included, for simplicity.

According to this scenario, one or more Petri dishes are inside the incubator, whose internal conditions are adjusted thanks to decentralized controller. During a test, system measures Petri dish impedance and elaborates it to obtain C_{DL}, R_{CT}, or R_{M}. Furthermore, the computer communicates with

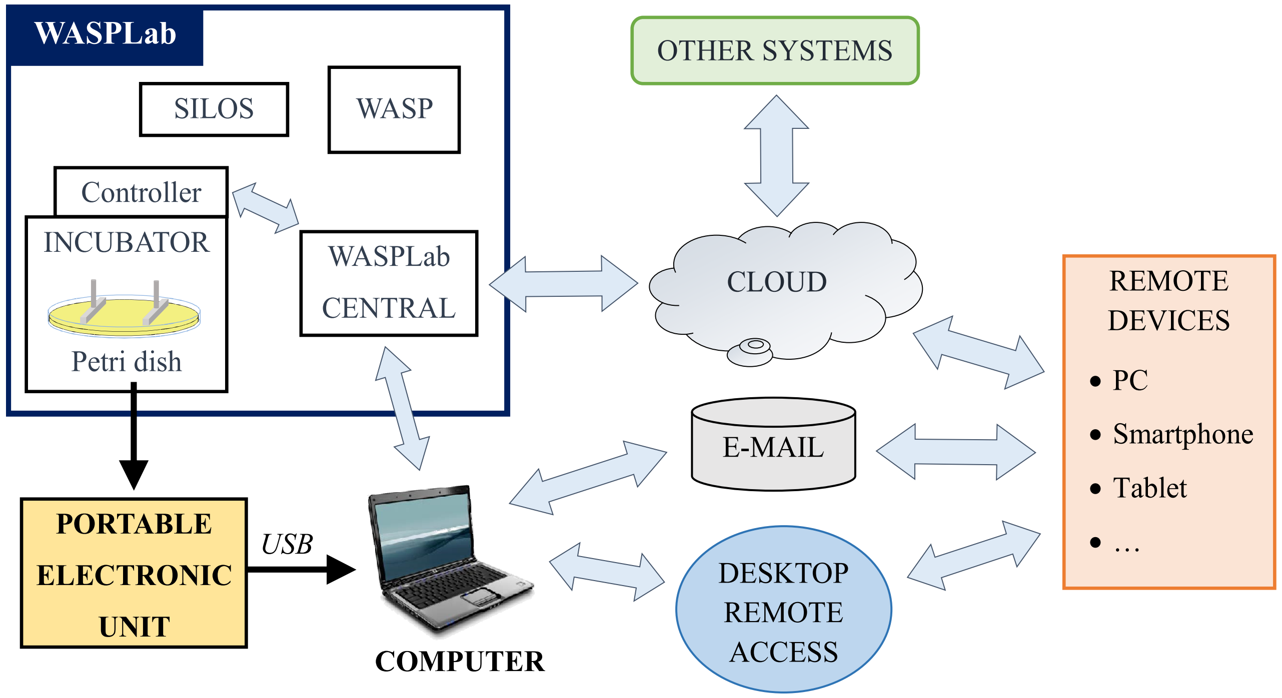


Fig. 2. Operation scenario: the measuring system working with the WASPLab automated platform.

WASPLab Central, exchanging information about the analysis on course (e.g., system state, occurring problems, diagnostics) and data regarding bacterial activity inside the analyzed Petri dishes. If necessary, both information and data are shared, through the cloud, with systems situated in other laboratories and any remote device (for instance, additional PCs, smartphones, and tablets) that has an Internet connection. In this way, they are available anywhere and at any time. Finally, through such devices, a distant user monitors analysis process by accessing computer's desktop remotely and receives a communication when an alarm is generated.

Another possible scenario includes system complete integration directly in the WASPLab, rather than an active collaboration between them. In this case, portable unit may become part of WASPLab electronics and computer functionalities may be covered by its user interface. Anyway, both alternatives go in the direction of an architecture meeting Industry 4.0 principles.

III. PERFORMED STUDY

Performed study consisted in a series of tests with the developed measuring system, in which we monitored bacterial growth in Petri dishes, while they were inside one of the WASPLab incubators.

A. Followed test protocol

Fig. 3 shows the block scheme of the experimental setup used for the study, which was carried out in COPAN Italia S.p.A., Brescia, Italy. Each test of the study followed the same protocol. Three Petri dishes were employed, which were filled with Tryptone Soy Agar culture medium. Then, two of them were inoculated with the same initial concentration C_0 of *S. Aureus* ATCC 6538. However, we considered different levels of C_0, i.e., in the order of $4.5 \cdot 10^8$ CFU/ml, $4.5 \cdot 10^7$ CFU/ml, and

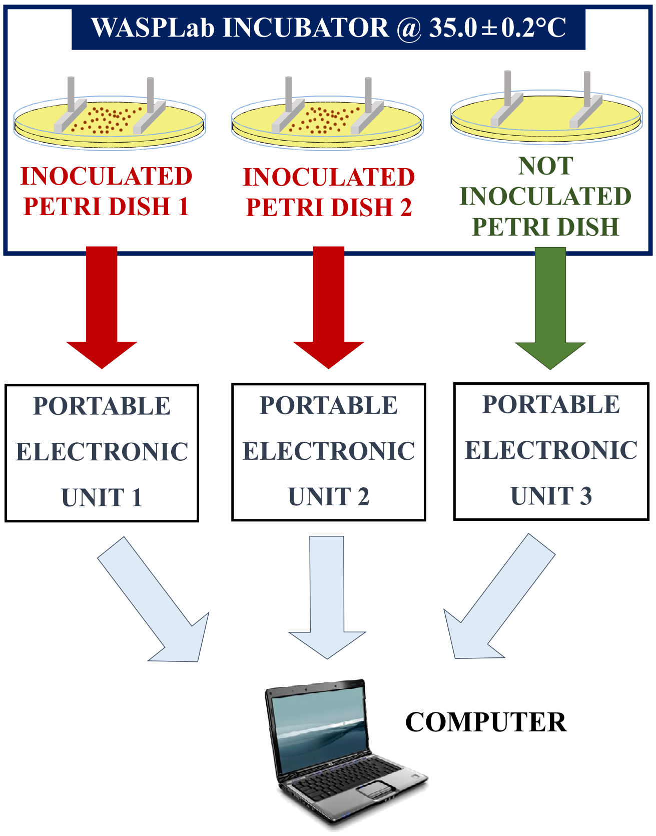


Fig. 3. Block scheme of the experimental setup used for the performed study.

Fig. 4. Measuring system working with the WASPLab during the study.

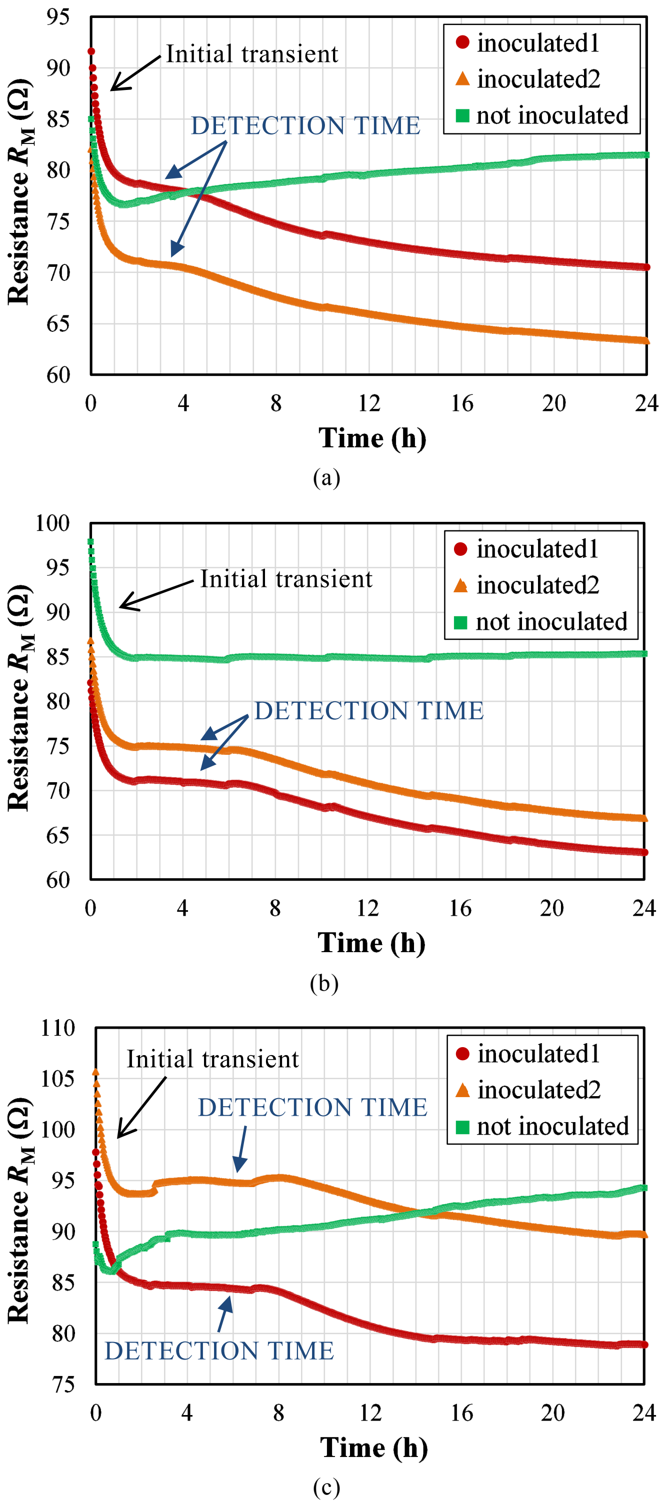
$4.5\cdot10^6$ CFU/ml, in different tests. Inoculation step was carried out in a safe location, at ambient temperature. On the contrary, third Petri dish was not inoculated, in order to have a reference for comparing the behavior characterizing inoculated dishes.

After this step, system was arranged for the measurement. As highlighted by Fig. 3, three portable electronic units were employed, one for each Petri dish, in order to test how they work together. Every unit was set in a way it could provide excitation waveforms to a Petri dish with an amplitude equal to 1 V_{pp}. Afterwards, the value of its internal gain factor was calculated by connecting a 100 Ω commercial resistor to its terminals and measuring resulting impedance.

Once system was ready to work, Petri dishes were stacked in one of the WASPLab incubators. For every test, incubator was turned on at least two hours before beginning the measurement session. In this way, internal temperature could be considered uniform inside its whole space. In addition, temperature was set to 35.0°C. In general, incubator controller kept it very stable, as it was always between 34.8 °C and 35.2 °C, except just after incubator was opened to position the Petri dishes. In fact, such action generated a perturbation (due to the difference with ambient temperature), which caused greater oscillations on it. Then, each Petri dish inside the incubator was connected to a portable electronic unit. Connection order was not the same for every test, since units are equal to each other.

Finally, computer program started running for continuous growth monitoring. In particular, the values of measurement parameters were set through its user interface, in a way to measure Petri dish impedance in correspondence of fixed frequencies f_1 = 50 Hz and f_2 = 150 Hz, every two minutes, for 24 hours from test beginning. Fig. 4 shows the system working with the WASPLab during a test of the study.

B. Results



(a)

(b)

(c)

Fig. 5. Resistance R_M as a function of time from tests with different levels of initial concentration C_0. (a) $C_0 = 4.5\cdot10^8$ CFU/ml. (b) $C_0 = 4.5\cdot10^7$ CFU/ml. (c) $C_0 = 4.5\cdot10^6$ CFU/ml.

Fig. 5 reports the time trend characterizing parameter R_M, resulting from the tests with initial concentration C_0 in the order of $4.5\cdot10^8$ CFU/ml (Fig.5a), $4.5\cdot10^7$ CFU/ml (Fig.5b), and $4.5\cdot10^6$ CFU/ml (Fig.5c). Represented curves have similar behaviors, despite the specific values assumed by R_M for the analyzed Petri dishes (standard deviation for values at time 0 is 8.4% of the corresponding mean value). This is especially true for the curves related to inoculated Petri dishes of the same test. All curves present an initial transient, which is due to medium temperature settling from ambient temperature (i.e., during inoculation step) to about 35°C (i.e., during incubation). Then, the curves related to not inoculated Petri dishes have a progressive increasing trend, which is most likely caused by medium partial drying. In fact, we always found water droplets on all Petri dish covers, at the end of every test. On the contrary, the curves referring to inoculated Petri dishes are characterized by a decrease during log phase, after a lag phase in which R_{M} is almost constant. This trend lasts until the end of the 24th hour (even though its rate diminishes gradually). Observed decrease is in agreement with what is found in the literature, since it derives from an increase in medium conductivity due to bacterial metabolism, which transforms medium weakly charged molecules into highly charged particles [4]. In general, results highlight that the measuring system successfully detects bacterial growth, when Petri dishes are inside WASPLab incubators, by noticing a variation in the observed parameters (R_{M} decrease in this case). As shown in Fig. 5, the change in curves slope between lag phase and log phase identifies its detection time, which is equal to three hours for $C_0 = 4.5\cdot10^8$ CFU/ml, four hours and a half when $C_0 = 4.5\cdot10^7$ CFU/ml, and six hours for $C_0 = 4.5\cdot10^6$ CFU/ml. Therefore, there is a relationship of inverse proportionality between detection time and the order of magnitude of C_0. This confirms what is reported in the literature as well [4].

IV. CONCLUSIONS

In this paper, we presented a study, in which we tested a previously developed measuring system while it was working with the WASPLab automated platform, from COPAN Italia S.p.A. In particular, we monitored bacterial growth in Petri dishes, while they were inside one of the WASPLab incubators. After having provided some information about the system, we illustrated test protocol of the study in detail. Then, we described the achieved results, which show that the system is able to detect the growth of bacterial cultures, not only in an *ad hoc* laboratory setting (as had been previously found [16]), but also directly in the field, discriminating among different levels of initial pathogen concentration.

In general, presented study suggests the possibility to add the measuring system to the WASPLab automated platform, once it is optimized. Possible scenarios include system complete integration directly in the WASPLab or an active collaboration between them. Both go in the direction of an architecture meeting Industry 4.0 principles. In fact, such solution would augment the quantity of data about bacterial growth, which would be exchanged with other connected systems and devices, to make them always available when necessary, also in remote locations. This would result in an increase of platform smartness. Anyway, regardless of the imagined scenario, the measuring system can help improving the automated analysis process that is performed with the WASPLab. In fact, bacterial growth monitoring could be achieved while Petri dishes are always inside the incubators. In addition, information about any growth phase can be acquired simultaneously from all analyzed dishes. Finally, system modularity and flexibility allow its easy reconfiguration according to context necessities. If the WASPLab is compared to a manufacturing machine, it can be concluded that all these aspects contribute to the realization of an architecture compliant with the model of the Factory of the Future.

ACKNOWLEDGMENT

The authors would like to thank Roberto Paroni and Giorgio Triva, from COPAN Italia S.p.A., for making the spaces and the instrumentation necessary for the study available. They thank also Stefania Fontana, always from COPAN Italia S.p.A., for her support in the execution of the experimental tests.

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