978-1-5386-2497-5/18/$31.00 ©2018 IEEE

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

Michele Bona, Paolo Bellitti, Emilio Sardini, Mauro Serpelloni
Department of Information Engineering
University of Brescia
Brescia, Italy
m.bona002@unibs.it

***Abstract*—As bacterial infections are still a risk for human health, the market offers different systems able to detect pathogens growth in biological samples. One of them is the WASPLab automated platform, by COPAN Italia S.p.A. Its operation is based on the processing of images of the Petri dishes containing a sample to analyze. In the present paper, we describe a study carried out on a previously developed system, whose operation relies on dish impedance measurement, to evaluate the possibility of its integration to the WASPLab. This would give a larger quantity of data about bacterial growth to the user and would optimize monitoring process. Through the developed 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 $4.5 \cdot 10^8$ CFU/ml, $4.5 \cdot 10^7$ CFU/ml, and $4.5 \cdot 10^6$ CFU/ml, respectively. Results highlight that the developed system could work together with the WASPLab, enhancing its monitoring performances.**

Keywords—dish impedance measurement; bacterial growth monitoring; detection time; 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, they can lead to severe diseases that put human health at risk. The situation is complicated by the fact that particular bacterial strains are able to resist to antibiotic therapies. 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 [2]-[4]. In this way, at least a discrimination between contaminated and sterile samples was possible, before sending them to specialized laboratories for complex and time-consuming tests. Such systems work with sensors whose operation is based on different physical principles [2]-[7]. Among the systems that are currently used, some examples are provided as follows. The BacTrac (Sy-Lab) performs a measurement of electric conductance of the bacterial culture medium, which changes because of pathogens activity [8]. Both the BacT/ALERT (BioMérieux) [9] and the BACTEC™ (Becton Dickinson) [10] detect a fluorescence variation, which is due to CO_2 production from bacterial metabolism. Then, the Biacore (GE Healthcare) exploits the principle of Surface Plasmon Resonance to detect the presence of bacteria on sensor surface [11]. Finally, the Thermal Activity Monitor (TA Instruments) evaluates bacterial growth from the heat generated by related chemical reactions, implementing isothermal microcalorimetry method [12].

An approach different from those followed by the previously mentioned systems is exploited by the WASPLab platform (COPAN Italia S.p.A.) [13]. WASP stays for Walk-Away Specimen Processor. Through this platform, 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 dish images. Furthermore, the WASPLab has several features that combine growth detection capabilities with high levels of automation and interaction with other smart systems. First, Petri dishes are inoculated and moved between incubator and image acquisition station in a completely automated way. Second, environmental conditions inside the incubator are controlled, to assure a proper bacterial growth. Then, images are elaborated by a processing software and can be visualized through a user interface. Finally, a station called WASPLab Central stores all images in a cloud and creates a communication between platform and collaborating systems.

However, the analysis process carried out by the WASPLab could be optimized. Firstly, the iterative movement of the Petri dishes between incubator and image acquisition station exposes them to a frequent change of environmental conditions, which could affect bacterial activity. Secondly, images of only two dishes can be taken at a time, since the platform is equipped with two image acquisition stations, each coupled with an incubator. In a previous work [14], we presented a system able to provide quantitative data about bacterial growth from Petri dish impedance measurement. In addition, we described the

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".

laboratory tests carried out in order to assess its capabilities in terms of measurement accuracy and growth detection. On the other side, the present paper illustrates a study, during which we tested such 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, in order to improve growth monitoring process. Paper following section provides basic information about the developed system. Then, third section describes the performed study, presenting either test protocol or achieved results.

II. The Measurement System

Fig. 1 shows a picture of the measurement system. Although its parts have been already described in detail in [14], 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 Petri dish, containing a medium that assures a proper growth of bacterial cultures. It is similar to those analyzed by the WASPLab. The only difference is that it is instrumented with an electrode-based sensor. 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. Such unit is contained in a box, which has dimensions 120 x 120 x 55 mm. It can be replicated for any Petri dish that is analyzed, increasing system modularity. Furthermore, the instrumented Petri dish is a disposable object that is connected to the electronic unit only during the analysis. Finally, the third part is a computer, which runs an interface program managing electronic unit operation and elaborating the impedance measurements. System final outputs provide information about bacterial growth. Besides impedance data, they are double layer capacitance C_{DL} and charge transfer resistance R_{CT}, which describe the behavior at electrode/ medium interface inside the instrumented Petri dish, and medium resistance R_M. The computer stores all the obtained data and presents them to the user, in real time. Then, developed system monitors bacterial growth autonomously, producing an alarm as it detects enough variation of C_{DL}, R_{CT}, or R_M. In addition, interface program permits to set 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.

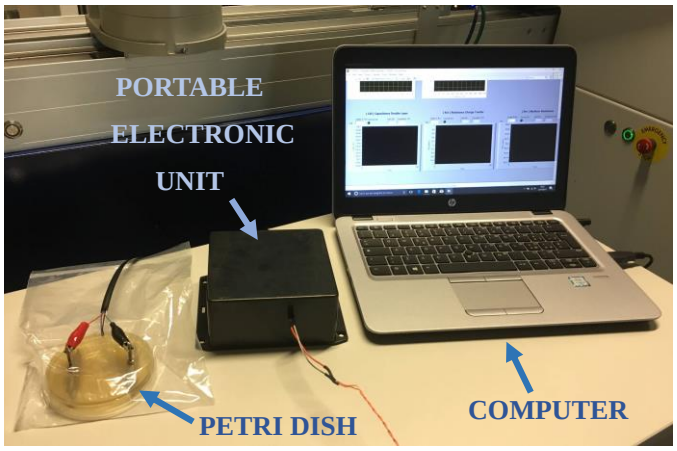

Fig. 1. The developed measurement system.

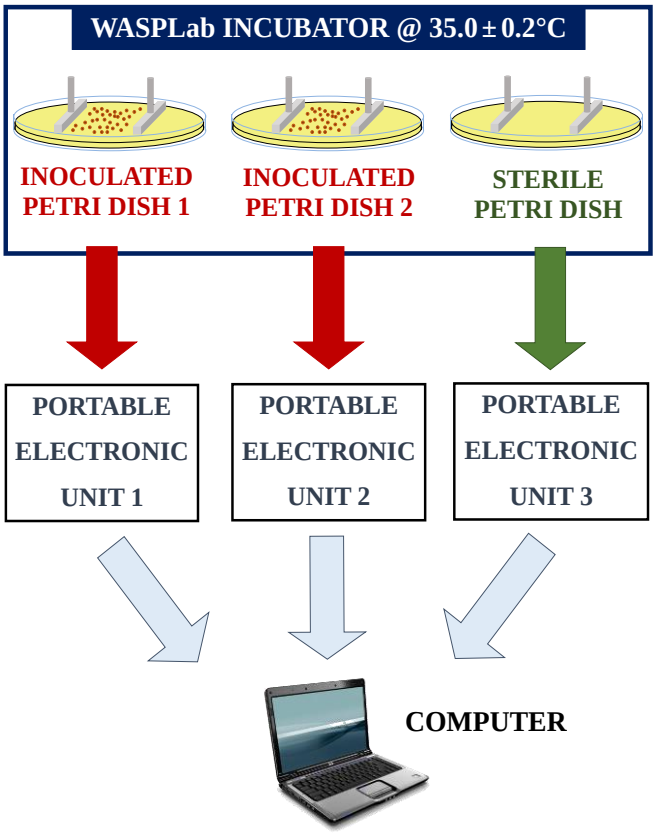

Fig. 2. Block scheme of the experimental setup used for the performed study.

III. Performed Study

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

A. Followed test protocol

Fig. 2 illustrates the block scheme of the experimental setup used for the study, which was carried out in COPAN Italia S.p.A. Each test composing this study followed the same protocol. Three Petri dishes were employed. They 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., $4.5 \cdot 10^8$ CFU/ml, $4.5 \cdot 10^7$ CFU/ml, and $4.5 \cdot 10^6$ CFU/ml, in different tests. Inoculation step was carried out in a safe location, at ambient temperature. On the contrary, third Petri dish was kept sterile, to have a reference for comparing the behavior characterizing inoculated dishes.

After inoculation step, system was arranged for the measurement. As highlighted by Fig. 2, three portable electronic units were employed, one for each Petri dish. Every unit was set in a way it could provide excitation waveforms to the corresponding 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.

Fig. 3. Measurement system working with the WASPLab platform.

Once system was ready to work, all 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, WASPLab control system 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 dish inside the incubator was connected to the terminals of 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. 3 shows the system working with the WASPLab platform during one of the tests.

B. Results

Fig. 4 reports the time trend characterizing resistance R_M, resulting from tests with initial concentration C_0 equal to $4.5 \cdot 10^8$ CFU/ml (Fig.4a), $4.5 \cdot 10^7$ CFU/ml (Fig.4b), and $4.5 \cdot 10^6$ CFU/ml (Fig.4c). Represented curves have similar behaviors, despite the particular values assumed by R_M for the analyzed Petri dishes. This is especially true for those related to inoculated dishes in 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 sterile dishes have a progressive increasing trend, which is most likely caused by medium partial drying. In fact, we always found water droplets on all dish covers, at the end of every executed test. On the contrary, the curves referring to inoculated 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

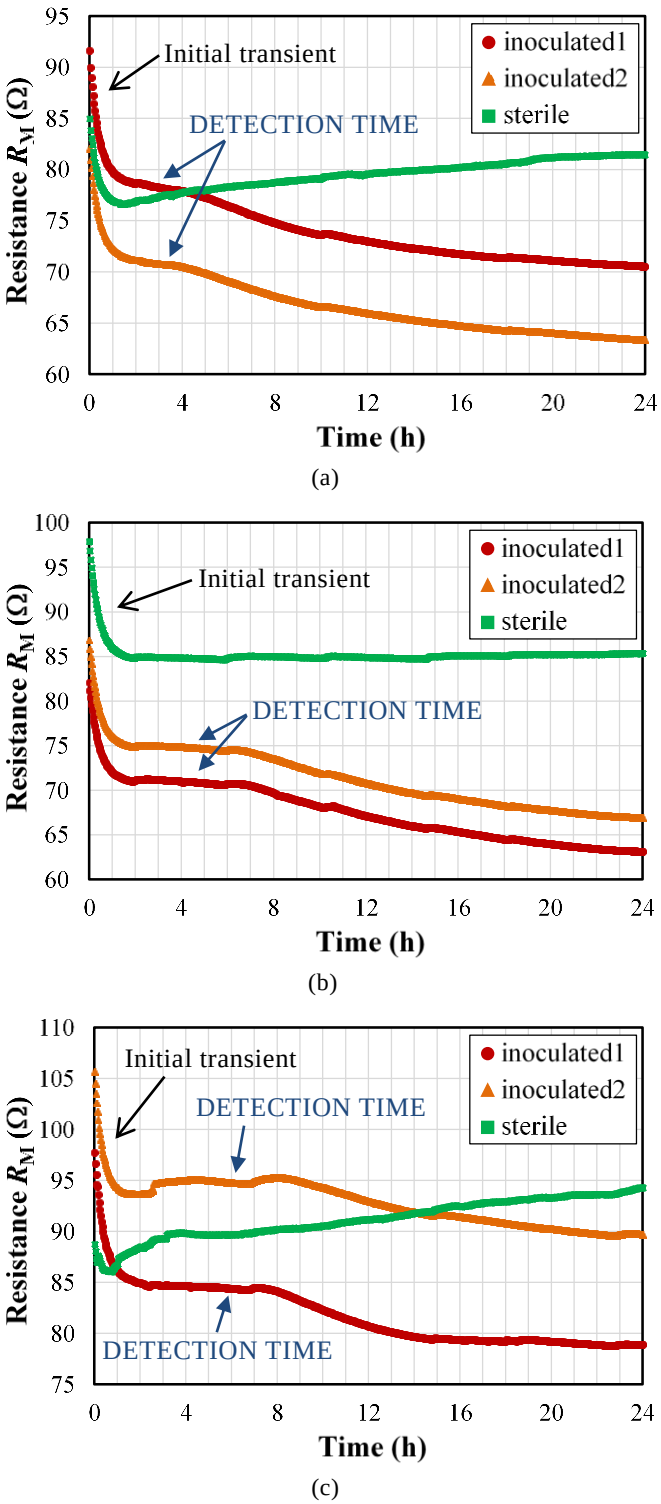

Fig. 4. Resistance R_M as a function of time from tests with different levels of initial concentration C_0: (a) $C_0 = 4.5 \cdot 10^8$ CFU/ml; (b) $C_0 = 4.5 \cdot 10^7$ CFU/ml; (c) $C_0 = 4.5 \cdot 10^6$ CFU/ml.

in medium conductivity due to bacterial metabolism, which transforms medium weakly charged molecules into highly charged particles [3]. In general, results highlight that 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. 4, the change in curves slope between lag phase and log phase identifies its detection time, which is equal to three hours for $C_0 = 4.5 \cdot 10^8$ CFU/ml, four hours and a half when $C_0 = 4.5 \cdot 10^7$ CFU/ml, and six hours for $C_0 = 4.5 \cdot 10^6$ 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 [3].

IV. Conclusions

In this paper, we presented a study, in which we tested a previously developed measurement 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 basic 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 bacterial growth, not only in an *ad hoc* laboratory setting (as had been previously found), but also directly in the field, discriminating among different levels of initial pathogen concentration.

In general, presented study suggests the possibility to add the system to the WASPLab automated platform, once it is optimized. Possible scenarios include system integration directly in the WASPLab or an active collaboration between them. Both go in the direction of an architecture meeting the Factory of the Future principles. In fact, this would augment the quantity of data about bacterial growth, which would be stored and exchanged through WASPLab Central, to make them always available when necessary. This would result in an increase of platform smartness. Anyway, regardless of the imagined scenario, the measurement system can help improving the automated analysis process that is performed with the WASPLab platform. 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.

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.

References

[1] "Preventing diarrhoea through better water, sanitation and hygiene," WHO Library Cataloguing-in-Publication Data, ISBN 978 92 4 156482 3, France, 2014.

[2] D. Ivnitski, I. Abdel-Hamid, P. Atanasov, and E. Wilkins, "Biosensors for detection of pathogenic bacteria," Biosens. Bioelectron., vol. 14, pp. 599-624, 1999.

[3] L. Yang and R. Bashir, "Electrical/electrochemical impedance for rapid detection of foodborne pathogenic bacteria," Biotechnol. Adv., vol. 26, pp. 135-150, 2008.

[4] J.H.T. Luong, K.B. Male, and J.D. Glennon, "Biosensor technology: Technology push versus market pull," Biotechnol. Adv., vol. 26, pp. 492-500, 2008.

[5] K. Warriner and A. Namvar, "Biosensors for Foodborne Pathogen Detection. Agricultural and Related Biotechnologies," in Comprehensive Biotechnology, 2nd ed., vol. 4, 2011, pp. 659-674.

[6] P. Arora, A. Sindhu, N. Dilbaghi, and A. Chaudhury, "Biosensors as inoovative tools for the detection of food borne pathogens," Biosens. Bioelectron., vol. 28, pp. 1-12, 2011.

[7] R.S. Burlage and J. Tillmann, "Biosensors of bacterial cells," J. Microbiol. Methods, vol. 138, pp. 2-11, Jul. 2017.

[8] G. Szita *et al*., "Detection of *Pseudomonas aeruginosa* in water samples using a novel synthetic medium and impedimetric technology," Lett. Appl. Microbiol., vol. 45, pp. 42-46, 2007.

[9] D.R. Murdoch and R.L. Greenless, "Rapid identification of *Staphylococcus aureus* from BacT/ALERT blood culture bottles by direct Gram stain characteristics," J. Clin. Pathol., vol. 57, pp. 199-201, 2003.

[10] J. Schroeter, I. Wilkemeyer, R.A. Schiller, and A. Pruss, "Validation of the microbiological testing of tissue preparations using the BACTEC™ blood culture system," Transfus. Med. Hemother., vol. 39, pp. 387-390, 2012.

[11] P.M. Fratamico, T.P. Strobaugh, M.B. Medina, and A.G. Gehring, "Detection of *Escherichia coli* O157:H7 using a surface plasmon resonance biosensor," Biotechnol. Tech., vol. 12, no. 7, pp. 571-576, Jul. 1998.

[12] O. Braissant, D. Wirz, B. Göpfert, and A.U. Daniels, "Use of isothermal microcalorimetry to monitor microbial activities," FEMS Microbiol. Lett., vol. 303, pp.1-8, 2010.

[13] http://products.copangroup.com/index.php/products/lab-automation/wasplab

[14] P. Bellitti, M. Bona, M. Borghetti, E. Sardini, and M. Serpelloni, "Flexible monitoring system for automated detection of bacterial growth in a commercial specimen processing platform," in Proc. 3rd IEEE International Forum on Research and Technologies for Society and Industry (IEEE RTSI), Modena, Italy, September 2017, pp. 207-212.