engineering proceedings

MDPI

Proceeding Paper

Detection of Escherichia coli and Staphylococcus aureus on sensors without immobilization by Impedance Spectroscopy

Oksana Gutsul [1, 2,*], David Rutherford [1], Marketa Barinkova [1], Vsevolod Slobodyan [3] and Bohuslav Rezek [1]

[1] Faculty of Electrical Engineering, Czech Technical University, Czech Republic; gutsul@bsmu.edu.ua; ruthedav@fel.cvut.cz; barinmar@fel.cvut.cz; rezekboh@fel.cvut.cz
2 Bukovinian State Medical University, Chernivtsi, Ukraine
3 Yuriy Fedkovich Chernivtsi National University, Chernivtsi, Ukraine; ;v.slobodyan@chnu.edu.ua
* Correspondence: gutsul@bsmu.edu.ua

Presented at the 10th International Electronic Conference on Sensors and Applications (ECSA-10), 15–30 November 2023; Available online: https://ecsa-10.sciforum.net/.

Abstract: The impedance spectroscopy method (AC f=4 Hz-8 MHz at a constant amplitude of 1 V) and Pt-IDE sensors were used to detect and monitor different concentrations (10^3, 10^6, 10^9 CFU/ml) of both live and dead bacteria cells (*Escherichia coli* and *Staphylococcus aureus*). The analysis of the impedance spectra shows difference in resistance with increasing concentration for both types of bacteria and the presence of characteristic changes in the frequency range 10-100 kHz. The presence of live bacteria led to a decrease in the impedance value compared to dead cells, the value of R_s+R_{ct} decreased about two times.

Keywords: impedance spectroscopy; IDE sensors; bacteria detection; Escherichia coli; Staphylococcus aureus

Citation: Gutsul, O.; Rutherford, D.; Barinkova, M.; Slobodyan, V.; Rezek, B. Detection of Escherichia coli and Staphylococcus aureus on sensors without immobilization by Impedance Spectroscopy. **2023**, *5*, x. https://doi.org/10.3390/xxxxx
Published: 15 November 2023

1. Introduction

Detection of bacteria is important in various fields, including health care, food safety, and environmental monitoring. Rapid and accurate identification of bacterial pathogens such as *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) is essential for public health and safety. Traditional methods of bacterial detection often involve time-consuming sample preparation steps, specialist personnel and equipment that involves complex analyses. In the last decade, more innovative methods have been developed that greatly simplifies the approach for real-time detection of bacteria [1–4], such as Electrical Impedance Spectroscopy (EIS).

Electrical impedance spectroscopy (EIS) is used to study various biological samples in suspension and is capable of characterizing the properties of biological samples [5–7]. Due to the native net negative surface charge on live bacteria [8], the native negative surface charge on live bacterial cells enables the detection and characterization by electrical impedance measurements. EIS uses the electrical properties of bacterial cells that enables detection and characterization by monitoring changes in electrical impedance. The impedance Z is the ratio of the applied voltage to the measured current and is a function of resistance (R), capacitance (C) and the applied frequency: $Z=V/I = R+1/j\ \omega\ C$ [7,9].

The metabolic activity of bacteria can be controlled by changes in the conductivity of the nutrient medium [10–12]. EIS has successfully been used to monitor changes related to adhesion, growth of bacteria and their behavior in real time [1-3]. Impedance-based detection of bacteria has several advantages over more traditional detection methods, such as low cost, versatility, and ease of implementation [5, 6]. However, very few works have been devoted to the direct detection of bacteria on interdigitated electrode (IDE)

sensors without bacterial immobilization using impedance spectroscopy [1,6,13]. Modern biosensors typically require immobilization of specific antibodies onto the surface of the sensor to offer high specificity but have a low sensitivity mainly due to low capture efficiency (<35%) even after careful optimization [2,14]. Therefore, the search for alternative methods of bacteria detection that do not depend on the immobilization of the electrode surface is still relevant.

In this paper, we review the principles, methodology, and applications of direct detection of *E. coli* and *S. aureus* on IDE sensors using immobilization-free impedance spectroscopy. We have proposed an approach for direct detection of bacteria on IDE sensors by measuring the change in impedance. This method avoids complex surface modification and immobilization stages, simplifying the detection process while maintaining high measurement sensitivity [6,9,11,13,15]. The presented method is capable of detecting the bacterial concentration (10^3 - 10^9 CFU/ml) in a short time (30 s) in a wide frequency range (4 Hz - 8 MHz) and demonstrated selectivity for two different types of bacterial cells (*E.coli* and *S. aureus*). We also demonstrate the ability to distinguish between living and dead cells. At the same time, the work processes for preparing the sensor surface are simplified, thereby increasing the economic efficiency of this method and reducing the need for specialist trained personnel. There is an obvious prospect of practical application for this method of selective detection of various types of bacteria.

2. Materials and Methods

2.1. Preparation of the Biological Samples (Gram-positive S. aureus and Gram-negative E.coli)

Gram-negative *E. coli* (CCM3954) and gram-positive *S. aureus* (CCM 3953) were stored at -20 °C in single-use vials and thawed at room temperature before use. A 1-in-10 dilution series was performed from the stock vial using sterile 0.9 % NaCl (Penta), and 500 µL of each dilution was added to petri dishes containing Mueller Hinton (MH) agar that were placed overnight in an incubator (37°C). The following day, a single colony was removed from MH agar plate and reconstituted in 5 mL MH broth and grown overnight at 37°C in an orbital shaker (150 rpm). After culturing, bacteria were centrifuged (13000 rpm at 10 min), to separate bacterial cells from MH broth and resuspended in sterile deionised water (DH_2O, (σ) = 0.1 µS/cm). This process was repeated a further two times to remove any residual MH broth. To obtain dead bacteria, the first wash step was preceeded with the additional wash using 99.9% ethanol and confirmed by the absence of growth on MH agar. Bacteria were then adjusted to McFarland's density 6.0 which is equivalent to 1.8 x 10^9 colony forming units per millilitre (CFU/ml) before sequential dilutionusing DH_2O to the desired concentrations (10^3, 10^6, 10^9 CFU/ml).

2.2. Preparation of the IDE sensor surface

Prior to use, the surface of the Pt-IDE sensor platform was cleaned with isopropanol, then rinsed with deionized water and dried under a stream of nitrogen.

2.3. Electrical Impedance Measurements

Electrical impedance spectroscopy (EIS) measurements with IDE (Au/Pt) sensors were made by using IM 3536 LCR meter and application software (Hioki) in the frequency range from 4 Hz up to 8 MHz. The sample was placed in a Faraday cage, the electrodes we fixed by a clamp, and connected to the LCR meter. Experimental Nyquist plots of the impedance $-Z_{im}=f(Z_{rel})$ were constructed to analyze the electron transport processes occurring at the interface of the Pt-IDE sensor and two types of bacteria cells (*E.coli* and *S.aureus*). All measurements were conducted at a temperature of 24±1ºC and the immersion sample volume was 1 ml. The general scheme of the proposed method for the detection of bacteria using impedance spectroscopy on IDE sensors is shown in Fig. 1.

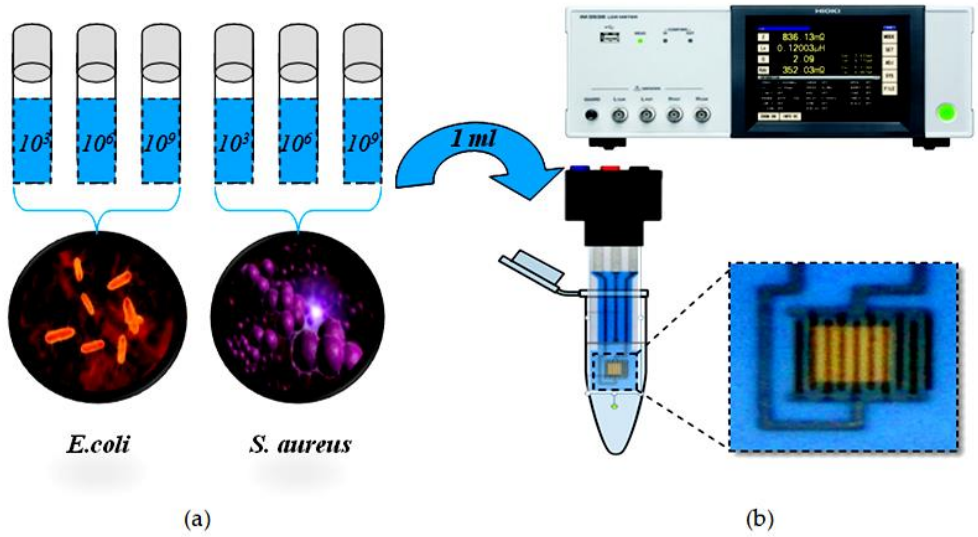

Figure 1. Schematic image of bacteria detection by EIS on IDE sensor: (a) preparation of different concentrations of bacteria; (b) IM 3536 LCR and IDE sensor (total view and zoomed measurement part).

3. Results and Discussion

3.1. Detection of bacteria cells. Characterization of Impedance Spectrum Data

Impedance spectroscopy wasperformed in deionized water (DH_2O) (non-Faraday EIS) with increasing E. coli concentrations (10^3, 10^6, 10^9 CFU/ml) in a frequency range between 4 Hz and 8 MHz at a formal voltage of 1 V. The effect of different concentrations of *E. coli* bacteriacells on DH_2O pH was previously measured (Fig. 2a). The growth of live and dead cells was monitored using agar plates for 24 hours at 37°C (Fig. 2b).

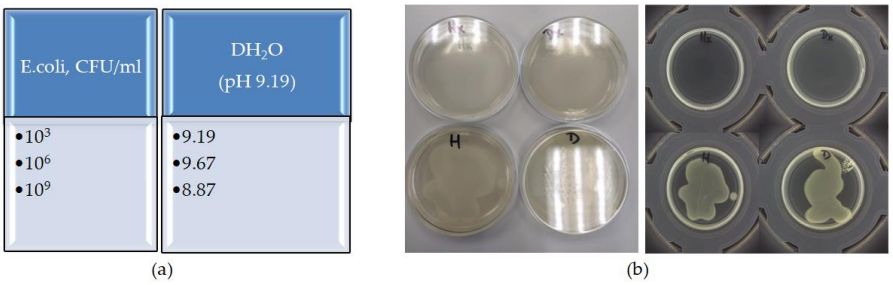

Figure 2. *E. coli* in DH_2O: (a) pH of *E. coli* for different CFU/ml; (b) Recultivated *E. coli* colonies (10^8 CFU/ml) (live (H/D) and dead (H_x/D_x) with HPLC (H) and DH_2O (D) on LB agar plate after incubation.

The Nyquist plots (Fig. 3) were fitted with an equivalent circuit (inset Fig. 3), showing that the R_{ct} is a relevant parameter that depends on the bacterial cell concentration. The Nyquist curves were fitted using the EIS analyzer software, and the best results were obtained with the equivalent circuit (inset of Fig. 3), where CPE is a constant phase element included in the circuit in parallel and in series with the charge transfer resistance R_{ct}, R_s is the solution resistance. A decrease in the charge transfer resistance R_{ct} was observed with an increase in the concentration of bacterial cells, both live (Fig. 3a) and dead (Fig. 3b) in deionized water.

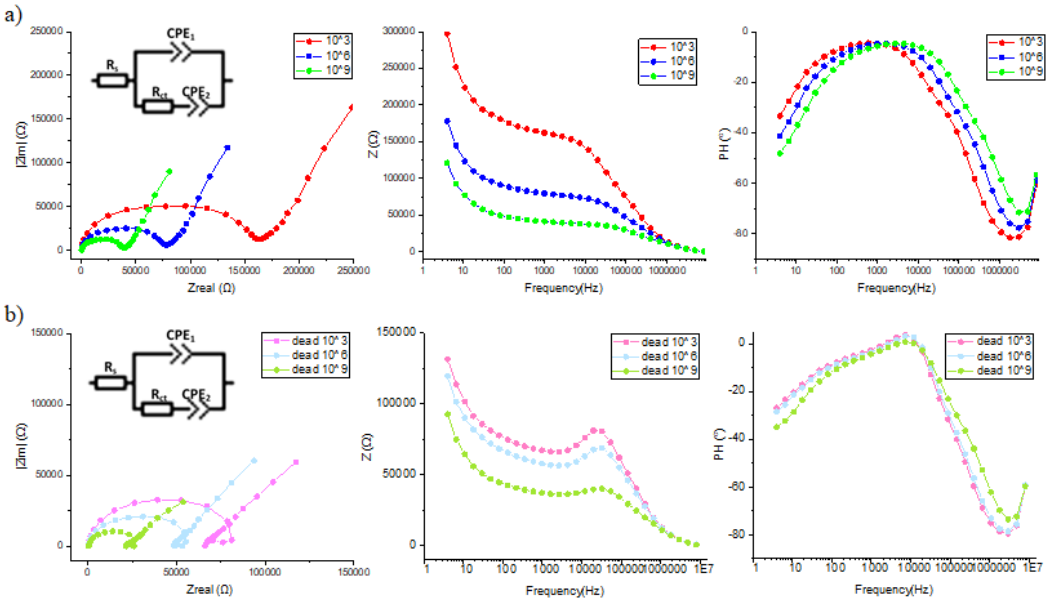

Figure 3. Impedance spectra (Nyquist and Bode plots) of E.coli (10^3, 10^6, 10^9 CFU/ml): (a) live and (b) dead bacteria.

The semicircle-shaped portion of the Nyquist plots obtained at high frequencies corresponds to the faradic transfer of electrons on the electrodes, while the spectrum obtained at low frequencies provides information on the diffusion process of transferring bacterial waste products in solution to the electrode surface. For dead *E. coli* bacteria, characteristic changes were observed at frequencies of 10-100 kHz, which were absent in the impedance spectra for live cells, which can serve as an identifier for distinguishing live cells from dead ones.

The obtained estimated parameters of charge transfer resistance, series resistance and for series and parallel CPE are shown in Table 1.

Table 1. Evaluated parameters for *E.coli* EIS (live and dead cells).

CFU/ml	Live						Dead					
	Rs, Ω ($\times10^{-14}$)	Rct, kΩ	CPE1 ($\times10^{-10}$)	n1	CPE2 ($\times10^{-7}$)	n2	Rs, Ω ($\times10^{-14}$)	Rct, kΩ	CPE1 ($\times10^{-11}$)	n1	CPE2 ($\times10^{-6}$)	n2
10^3	9.31	166	5.55	0.74	5.86	0.69	8.69	70.24	1.37	0.99	1.88	0,60
10^6	9.01	80.53	7.04	0.74	7.59	0.71	8.64	50.52	4.05	0.93	1.85	0.61
10^9	8.79	41.08	8.86	0.73	9.56	0.72	7.26	22.67	1.33	1.00	4.08	0.50

.

For dead cells, a decrease in the CPE values by an order of magnitude is observed (Table 1). Moreover, the value of CPE1 decreases by an order of magnitude, which is associated with a change in the capacitive properties of the membranes of dead cells. CPE2, responsible for the change in mass transfer in solution, increases by an order of magnitude, indicating an increase in ion diffusion in solution due to changes in osmotic pressure inside and outside the dead cells. There is a significant decrease in resistance for suspensions with dead *E. coli* cells. For comparison, the impedance spectra for live and dead cells with a concentration of 10^3 and 10^6 CFU/ml are shown below in one plot (Fig. 4a).

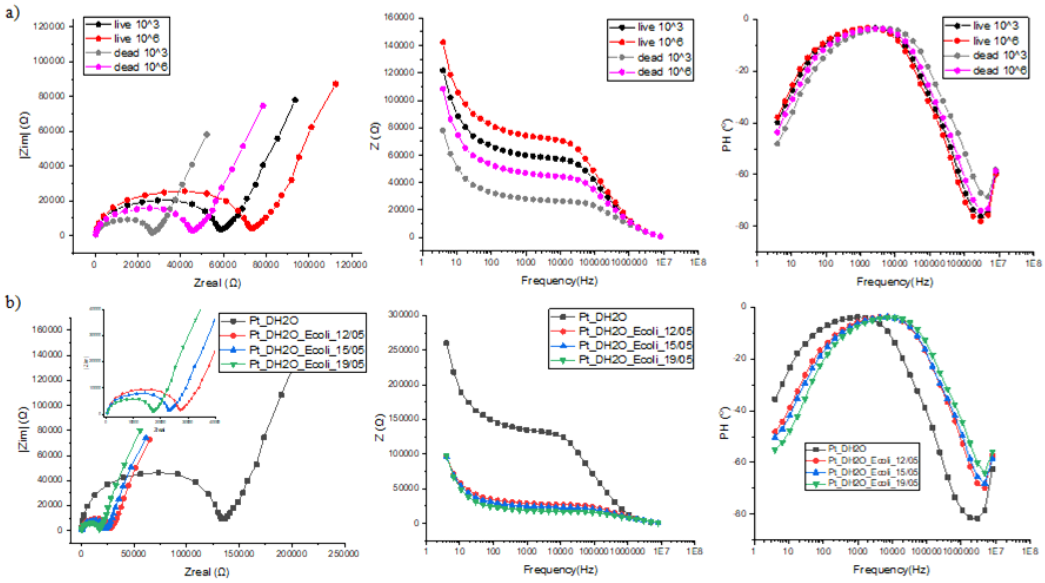

Figure 4. Comparison of Impedance spectra of *E.coli* (Nyquist and Bode plots): (a) *E.coli* live and dead; (b) *E.coli* 10^8 CFU/ml (during 1st, 4th and 8th day) and DH_2O.

Impedance measurements were also performed for live *E. coli* 10^8 CFU/ml cells in DH_2O on 1st, 4th and 8th day after suspension preparation. There was a tendency to decrease the charge transfer resistance with increasing storage time of the suspension, which is associated with increase of bacteria waste products. For comparison, the impedance spectrum for pure deionized water is shown (Fig. 4b – black curve). It is obvious that the increase in the electrical conductivity of the suspension is due to the presence of bacterial cells.

To compare the impedance spectra of *E. coli* and *S.aureus* in deionized water, suspensions with a concentration of 10^9 CFU/ml were chosen. The Nyquist and Bode curves are shown in Fig. 5a,b.

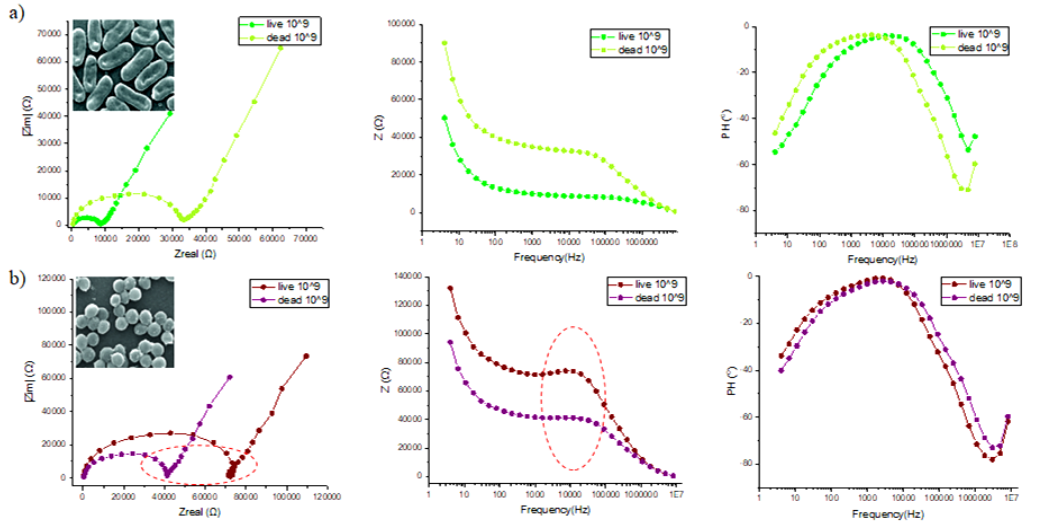

Figure 5. Nyquist and Bode plots of Impedance spectra live/dead: (a) *E.coli*; (b) *S.aureus*.

The obtained estimated parameters of the charge transfer resistance R_{ct} and series resistance R_s, as well as the values of the CPEs included in parallel and in series with the charge transfer resistance R_{ct} are given below in Table 2.

Table 2. Evaluated parameters for live and dead bacterial cells.

10^8 CFU/ml	Rs, Ω (×10^{-3})	Rct, kΩ	CPE1	n1	CPE2 (×10^{-6})	n2
			E.coli			
Live	1.60	9.21	8.86×10^{-09}	0,70	2.26	0,70
Dead	1.62	32.70	5.39×10^{-10}	0.77	1.35	0.72
			S.aureus			
Live	9.59	73.46	1.36×10^{-10}	0.85	1.23	0.70
Dead	9.58	58.37	1.73×10^{-10}	0.83	1.40	0.67

.

Opposite dependencies of the change in charge transfer resistance and the obtained values of the total impedance for live and dead cells for the two types of bacteria are observed. This difference is obviously related to the structural features of these types of bacteria and their size. *S.aureus* are spherical cells that tend to form larger agglomerates whereas *E.coli* are rod-shaped cells and preferentially exist as individual cells. For *S.aureus*, increase CPE1 (the capacity of the double layer C_{dl}) is due to changing in bacterial cells membranes structure.

4. Conclusions

The proposed method of selective detection of bacterial cells can be used to differentiate between two types of bacteria, specifically *E.coli* and *S.aureus*, as well as qualitatively characterize their physiological state i.e., dead or live, and to estimate their concentration in samples with an unknown number of bacteria per unit volume.

5. Patents

Acknowledgments: This work has been supported by the TACR project TM03000033 (TACOM) and by the MEYS project CZ.02.01.01/00/22_008/0004596 (SenDISo).

Conflicts of Interest: The authors have no financial/commercial Conflict of Interest.

References

[1] M. Mallén-Alberdi: N. Vigués, J. Mas, C. Fernández-Sánchez, A. Baldi, Impedance spectral fingerprint of E. coli cells on interdigitated electrodes: A new approach for label free and selective detection, Sensing and Bio-Sensing Research. 7 (2016) 100–106. https://doi.org/10.1016/j.sbsr.2016.02.001.

[2] R. Wang, J. Lum, Z. Callaway, J. Lin, W. Bottje, Y. Li, A Label-Free Impedance Immunosensor Using Screen-Printed Interdigitated Electrodes and Magnetic Nanobeads for the Detection of E. coli O157:H7, Biosensors. 5 (2015) 791–803. https://doi.org/10.3390/bios5040791.

[3] H. Yang, H. Zhou, H. Hao, Q. Gong, K. Nie, Detection of Escherichia coli with a label-free impedimetric biosensor based on lectin functionalized mixed self-assembled monolayer, Sensors and Actuators B: Chemical. 229 (2016) 297–304. https://doi.org/10.1016/j.snb.2015.08.034.

[4] A. Ahmed, J.V. Rushworth, N.A. Hirst, P.A. Millner, Biosensors for Whole-Cell Bacterial Detection, Clin Microbiol Rev. 27 (2014) 631–646. https://doi.org/10.1128/CMR.00120-13.

[5] M. Barreiros Dos Santos, C. Sporer, N. Sanvicens, N. Pascual, A. Errachid, E. Martinez, M.-P. Marco, V. Teixeira, J. Samiter, Detection of pathogenic Bacteria by Electrochemical Impedance Spectroscopy: Influence of the immobilization strategies on the sensor performance, Procedia Chemistry. 1 (2009) 1291–1294. https://doi.org/10.1016/j.proche.2009.07.322.

[6] S. Brosel-Oliu, N. Abramova, N. Uria, A. Bratov, Impedimetric transducers based on interdigitated electrode arrays for bacterial detection – A review, Analytica Chimica Acta. 1088 (2019) 1–19. https://doi.org/10.1016/j.aca.2019.09.026.

[7] P. Swami, A. Sharma, S. Anand, S. Gupta, DEPIS: A combined dielectrophoresis and impedance spectroscopy platform for rapid cell viability and antimicrobial susceptibility analysis, Biosensors and Bioelectronics. 182 (2021) 113190. https://doi.org/10.1016/j.bios.2021.113190.

[8] J. Jira, B. Rezek, V. Kriha, A. Artemenko, I. Matolínová, V. Skakalova, P. Stenclova, A. Kromka, Inhibition of E. coli Growth by Nanodiamond and Graphene Oxide Enhanced by Luria-Bertani Medium, Nanomaterials. 8 (2018) 140. https://doi.org/10.3390/nano8030140.

[9] K. Kivirand, M. Min, T. Rinken, Challenges and Applications of Impedance-Based Biosensors in Water Analysis, in: T. Rinken, K. Kivirand (Eds.), Biosensors for Environmental Monitoring, IntechOpen, 2019. https://doi.org/10.5772/intechopen.89334.

[10] M.G. Massimo Lanzoni, Data Transformation Algorithm for Reliable Bacterial Concentration Detection Using the Impedance Method, J Electr Eng Electron Technol. 03 (2014). https://doi.org/10.4172/2325-9833.1000111.

[11] M. Cimafonte, A. Fulgione, R. Gaglione, M. Papaianni, R. Capparelli, A. Arciello, S. Bolletti Censi, G. Borriello, R. Velotta, B. Della Ventura, Screen Printed Based Impedimetric Immunosensor for Rapid Detection of Escherichia coli in Drinking Water, Sensors. 20 (2020) 274. https://doi.org/10.3390/s20010274.

[12] A. Kundu, T. Ausaf, P. Rajasekaran, S. Rajaraman, Multimodal Microfluidic Biosensor With Interdigitated Electrodes (IDE) And Microelectrode Array (MEA) For Bacterial Detection And Identification, in: 2019 20th International Conference on Solid-State Sensors, Actuators and Microsystems & Eurosensors XXXIII (TRANSDUCERS & EUROSENSORS XXXIII), IEEE, Berlin, Germany, 2019: pp. 1199–1202. https://doi.org/10.1109/TRANSDUCERS.2019.8808696.

[13] V. Singh, V. Rawal, S. Lakhanpal, P. Jain, S. Dahiya, C.C. Tripathi, IMMOBILIZED BACTERIOPHAGE USED FOR SPECIFIC DETECTION OF E.COLI USING ELECTROCHEMICAL IMPEDANCE SENSING, International Journal of Pharmaceutical Sciences and Research. 6 (n.d.).

[14] A.G. Gehring, D.M. Albin, A.K. Bhunia, S.A. Reed, S.-I. Tu, J. Uknalis, Antibody Microarray Detection of *Escherichia c oli* O157:H7: Quantification, Assay Limitations, and Capture Efficiency, Anal. Chem. 78 (2006) 6601–6607. https://doi.org/10.1021/ac0608467.

[15] A. Al-Jumaili, M.A. Zafar, K. Bazaka, J. Weerasinghe, M.V. Jacob, Bactericidal vertically aligned graphene networks derived from renewable precursor, Carbon Trends. 7 (2022) 100157. https://doi.org/10.1016/j.cartre.2022.100157.

Disclaimer/