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A new Bacteria-based Electrochemical Impedance water toxicity biosensor

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ABSTRACT

This article presents a biosensor prototype designed for early risk warning in freshwater quality assessment. Its principle lies in estimating the overall biological impact of large-scale contamination due to chemical accidents. The electrical impedance measurement principle is based on the hypothesis that the ionic concentration of solutions containing centrifugated bacteria resuspended in a deionised water, and exposed to pollutants, is linked to perturbation induced in bacterial cell walls. FEM simulations with a semi-analytical model were used to obtain the expected electrical response for control bacterial solutions, which was subsequently analysed using Electrochemical Impedance Spectroscopy (EIS) and Distribution of Relaxation Time (DRT). Non-toxicity for bacteria of CuSn and Cu electrodes was verified within the experience parameters. EIS experimental results for bacterial solutions exposed to an anthelminthic (Ivermectin) and an antibiotic (Ampicillin), show that it is possible to discriminate the polluted samples samples under 30 minutes by measuring the changes in conductivity. DRT data analysis allowed discrimination of polluted samples through tau-shifts. It also proved a powerful tool to analyse a specific electrochemical process, and on simulated data, it showed the potential to identify and study electrochemical processes (medium vs cell wall) involved. EIS on Ivermectin- and Ampicillin-exposed suspensions, reveals dose-dependent resistance decreases (Ampicillin 10/20/100µgmL−1: 2.01±0.40kΩ/1.62±0.02kΩ/0.89±0.08 kΩ; Ivermectin 15/45µgL−1:3.89±0.23kΩ/2.70±0.27kΩ). DRT is able to discerns processes via τ-shifts (Alive–Heat Δτ ≈ 2.0±0.9µs; Ampicillin 10/20/100µgmL−1:0.9/0.7/0.1µs; Ivermectin (15/45µgL−1:1.2/0.9µs). In conclusion, results obtained with this biosensor prototype validate our assumption, demonstrating the effectiveness of probing solution conductivity as an indicator of cell viability.

INTRODUCTION

The protection of water resources against industrial accidents is a major global concern. High-profile chemical incidents, such as the Lubrizol plant fire in France in 2019 (Jennifer *et al.*, 2024) which had critical consequences on the inhabitants and the environment during several days, have highlighted the inability of current monitoring strategies to detect sudden toxic spills in real-time. Therefore, establishing rapid early-warning systems is a major priority to manage these unpredictable, high-intensity contamination peaks.

Current water toxicity sensing methods are usually based on chemical or optical solutions (Campanale *et al.*, 2021; Mauriz *et al.*, 2006) but these methods are costly and time consuming (ISO, 1995; Justino *et al.*, 2015). Real-time sensors permitting fast and efficient responses are scarcely deployed. To reach an efficient protection, sensors combining biocomponents and transducers are emerging (Hassani *et al.*, 2017). These sensors, known as biosensors, convert pollutants biological effects directly into a

measurable signal, offering rapid, sensitive and inexpensive in-situ monitoring. Among other biocomponents (such as antibodies, phages or DNA), bacteria cells have the advantage to be easy to grow and genetically modifiable (Mehrotra et *al.*,2016).

In this context, this paper presents the development of an impedancemetric biosensor prototype without functionalization using bacteria as bioreceptors. The aim of this new biosensor is to overcome the shortcomings of current sensors, presented earlier. Note that the goal is to provide alerts, not to look for selectivity, which allows to design a simple and robust solution.

Our working hypothesis is that the ionic diffusion between the bacteria cytoplasm and the external environment during an accidental pollution is modifying the membrane permeability of bacteria. Considering the minimum resistance that is measurable by our setup, it is possible to tune the used medium conductivity for the bacterial solutions before exposure for a given sample volume and to detect electrical charge variations as an increase in the overall conductivity of the solution. To test this assumption, *Escherichia coli* bacterium has been chosen because it is widely studied and easy to grow. Relevant and representative pollutants were selected, and it must be noted that they do not perturbate the overall conductivity of the solution.

To introduce our analysis methodology, the first section presents the materials and methods, including the theoretical elements underlying the sensor physical principle, as well as the basics of the Distribution of Relaxation Times (DRT) algorithm. The results section begins with a validation of the measurement setup through a study checking that there is no influence of the electrodes on the cell wall integrity under our experimental conditions. Then, a semi-analytical model was used as a basis to simulate the electrical response of bacterial control solutions, comprising either alive or treated bacteria, with a Finite Element Model (FEM). EIS experimental results for a bacterial solution exposed to an anthelminthic and an antibiotic are shown, along with control solutions used as a baseline for comparison. Before concluding, a complementary study of these data using DRT illustrates the power of this data processing tool to analyse each specific electrochemical process involved.

I. MATERIALS AND METHODS

A. Electrical Impedance spectroscopy

Electrical Impedance Spectroscopy (EIS) is a simple, non-destructive technique that characterizes materials and systems by measuring an impedance across a frequency spectrum. This involves applying an alternating current (AC) signal and analysing the complex impedance, $Z=V/I$, from the voltage V response to the applied current I. In our experiments, an Agilent Precision Impedance Analyzer AD4294A, calibrated close to the electrodes, was used to measure Z for various solutions with a 100 mV AC excitation amplitude. The setup is shown on Figure 1. Every measurement has been done in the β dispersion region between 1 kHz up to 30 MHz (Heileman *et al.*, 2013). This bandwidth allows to circumvent electrode polarisation phenomena that lead the impedance response below 1 kHz and the need for more complex electrode configuration usually employed for lower frequencies.

Within Macdonald's framework (Macdonald *et al.*, 1992), water impedance is modelled with *RC* networks to resolve distinct electrochemical processes. In this work, we focus on the medium electrical response where experimental data can be fitted with a first-order lowpass RC modelling circuit (a single RC cell) using a standard non-linear regression method (CNLS). The fitted resistance (Ω) links the medium's conductivity in the *RC* representation, lower R indicating higher ionic conductivity for a given cell constant. It is thus serving as a practical measure of charge transport in water.

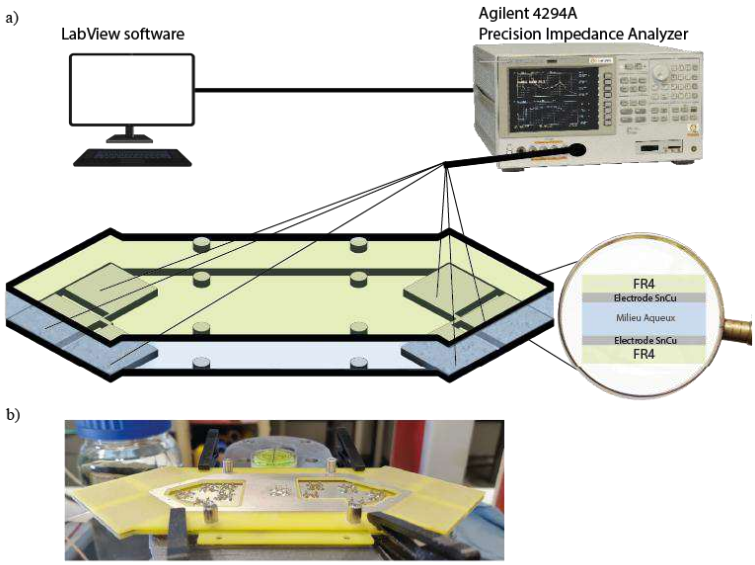


FIGURE 1. Experimental setup and electrode design. Electrodes are 25 x 25 mm^2, with an 800 µm gap, giving a 500 µL volume **(a)** Schematic diagram of the experimental layout showing the signal information process and electrodes. **(b)** Photograph of the fabricated electrodes on classical printed circuit boards (PCB-FR4).

B. Distribution of Relaxation Times (DRT)

DRT is a technique representing impedance as an infinite number of extremely small differential *RC* parallel circuits in series (Wan *et al.*, 2015; Ciucci *et al.*, 2015; Liu *et al.*, 2020). DRT analysis has been applied in this work on impedance data to translate the frequency-domain EIS spectra to a relaxation time-based function *γ(τ)*, expecting that it will facilitate the detection of the medium conductivity modification due to the perturbation of the bacteria cell-walls.

The time constant of a typical *RC* parallel circuit is $\tau = RC$. R_∞ represents the series resistance at the highest frequency of the acquired spectrum. DRT gives a graphical information of the statistical distribution of *RC* couples by means of the relaxation time distribution function $\gamma(\tau)$. All RC parallel circuits are considered in the system to obtain the formula (1).

$$Z_{DRT}(f) = R_\infty + \int_0^\infty \frac{\gamma(\tau)}{1 + i2\pi f\tau} d(\tau) \qquad (1)$$

C. Modification of a weakly conductive medium by bacteria: Estimating the number of charges in the vicinity of a bacterium

Our working hypothesis rests on the existence of cytoplasm ion diffusion to the external medium when a bacterium is treated. Since the exposed bacteria are suspended in deionised (DI) water, we suppose that the external medium is weakly conductive and that the treatment likely increases membrane permeabilization (Cass *et al.*, 1970; Lambert, 2002). In this case, when a treatment is applied to the bacterium, an increase of the medium conductivity will be observed.

In order to anticipate the number of charges impacting the overall conductivity of a solution composed of bacteria suspended in a weakly conductive medium, we first need to determine the order of magnitude for the number of resulting charges in the immediate vicinity of a bacterium in these conditions.

The electrostatic potential *ψ(r)* describes the potential variation in a liquid medium around the bacterium cell wall. From this cell wall, the measurable value in electrophoresis is the zeta potential V_ζ, often used as a proxy for surface charge. This potential is generally $V_\zeta = -50$ mV (Wilson *et al.*, 2001; Poortinga *et al.*, 2002; Li *et al.*, 2004). The spatial extent of electrostatic interactions is governed by the Debye length λ_D, which decreases as the ionic strength increases. For an electrolyte, we can write : $\lambda_D = \sqrt{\frac{\varepsilon_0 \varepsilon_r k_B T}{2 N_A e^2 I}}$, with T the temperature, k_B the Boltzmann constant and $I = \frac{1}{2}\sum_i C_i Z_i^2$ the ionic strength, C_i and Z_i representing concentration and valence of an ion, respectively.

Estimation of the number of charges in the outer vicinity of a bacterium is based on the Gouy-Chapman-Stern electric double-layer model (Stern, 1924). Two contributions are distinguished: the Stern layer, where ions are adsorbed to the surface, and the diffuse layer, where mobile ions are distributed by electrostatic forces.

The charge density in the Stern layer follows the relationship $\rho_{\text{Stern}} = \varepsilon_0 \varepsilon_r V_\zeta / 0.1nm$ in the linear approximation. The charge density in the diffuse layer is written $\rho_{\text{Diffuse}} = -\varepsilon_0 \varepsilon_r V_\zeta / \lambda_D^2$. The potential decreases from the surface according to $\psi(r) = V_\zeta e^{-r/\lambda_D}$, where r is the radial distance to the surface.

To model an *E. Coli* bacterium, we consider a cylinder: 2 µm in length and 0.5 µm in diameter. In a 5 g L^{-1} NaCl solution, the ionic strength is $I = 85.6$ mol m^{-3} and the Debye length $\lambda_D = 1.04 \times 10^{-9}$ m; integrating ρ_{Stern} over *S* gives an order of magnitude of around 6×10^6 charges for the Stern layer, and integrating ρ_{Diffuse} over the volume $S\lambda_D$ gives around 2.0×10^6 charges in the diffuse layer.

These charge densities increase as $|V_\zeta|$ increases and as λ_D decreases, so with increasing ionic strength. In dilute media, λ_D lengthens, the local density decreases, but the electrostatic influence extends further. In a concentrated medium, the double layer compresses and charges pack close to the wall. Thus, these values represent a first-order approximation and generally underestimate the role of transmembrane potential and complex ionic chemistry, as in this case only Na^+ and Cl^- ions are considered. Nevertheless, it provides an approximate of the charges brought by the bacterium to the medium, essential for simulation.

D. Preparation of Cu/CuSn Electrodes

Two kinds of electrode pairs have been used for this experiment. Both are covered with an 18 µm Cu layer but one pair also includes a top layer of 1 to 2 µm SnCu (total of 99.7% Sn and 0.3% Cu). Both have been fabricated to have a surface of 25 × 25 mm². Two 800 µm ±10 µm-thick polished glass spacers are used to ensure parallel plate configuration. Prior to each test, the electrode pair is immersed in ethanol and rinsed for disinfection and cleaning. The solution to be measured is injected between the electrodes using a (1000 µL ± 1 µL) pipette and held in place thanks to capillary forces.

E. Preparation of *E. coli* cultures

E. Coli (CIP 103931 Pasteur Institute) were maintained on glycerol protecting beads (Fisher Scientific, United Kingdom) at -80°C. For experimental tests, 20 mL of LB (NaCl 5 g L^{-1}) was aseptically inoculated with a single bead and incubated at 37°C for 16 h. After the incubation period, the culture contained 1×10^9 CFU mL^{-1} (Colony-Forming Unit). Different concentrations have been obtained by dilution in DI water.

F. Sample preparation for electrode toxicity test

Three *E. Coli* cultures ($\cong 10^7$ CFU mL^{-1}) were used to test electrode toxicity for both electrode kinds. For each culture, 500 µL were pipetted between each electrode pair and exposed for 40 minutes (at $T = 25$ °C), with no noticeable evaporation. After this time, samples were removed from the electrodes and analysed by fluorescence microscopy using a counting chamber and live/dead cell viability stains (Thermoficher SYTO9/Propidium Iodide).

G. Sample preparation for EIS measures

Three different populations were used to prepare the different samples, thus having three replicates. Each one was selected at the end of the exponential growth phase, then centrifugated and resuspended in DI water three times ($\sigma = 1 \times 10^{-4}$ S m^{-1}, $\varepsilon_r = 78$) obtaining a bacterial concentration of approximately 10^8 CFU mL^{-1}. Despite the hypoosmotic challenge, the cell walls of viable bacteria operate as a stabilizing mechanical unit, ensuring they remain structurally intact and protected from lysis (Deghelt et al., 2025). Each solution was then divided in as many samples as necessary for the different planned tests (Alive and Heat-Treated controls plus pollutant-treated samples). Heat-Treated solutions were heated at 90 °C for 20 minutes. In order to stay coherent with other publications we used the Alive tag for viable bacteria.

Pollutants chosen to test the biosensing principle were the antibiotic Ampicillin and the anthelminthic Ivermectin. Ampicillin is selected for its bactericidal properties and ability to disrupt bacterial cell wall synthesis (Chopra *et al.*,1963). Literature about the effect of Ivermectin on bacteria is very scarce. Studies on the subject (Ashraf *et al.*, 2018) suggest that Ivermectin has a bacteriostatic effect and no specific interaction with cell walls. The minimum inhibitory concentration (MIC) was determined to be 4 µg mL^{-1} for Ampicillin and 40 µg L^{-1} for Ivermectin respectively. Experimental testing was conducted at concentrations of 10 µg mL^{-1}, 20 µg mL^{-1} and 100 µg mL^{-1} for Ampicillin, and 15 µg L^{-1} and 45 µg L^{-1} for Ivermectin. We have first checked that both pollutants do not interfere in EIS measurements, and indeed they are non-conductive. The effects of Ampicillin and Ivermectin were monitored over 180 minutes, with impedance measurements taken at $t = 0$, 60, 120 and 180 minutes.

II. RESULTS AND DISCUSSION

A. Electrode toxicity

Copper was primarily selected for electrode fabrication due to its cost-effectiveness, simplicity of implementation, and widespread use as a standard electronic material in PCB. In literature, bulk copper and Cu-rich alloys are considered to have a bactericide effect (Noyce *et al.*, 2006).

However, in our experimental setup, time of contact between bacteria and electrodes is significantly smaller, since a sample measure needs less than 5 minutes, and measured volumes are significantly bigger (500 µL). Thus, we chose to check if electrodes used had any notable effect within our experimental setup, using the sample protocol described in section I.F.

Metal	Alive [unit]	Dead [unit]	Ratio Dead/Alive	Concentration CFU mL^{-1}
Cu	520	6	< 2%	$2{,}6 \times 10^7$
Cu	557	11	< 2%	$2{,}8 \times 10^7$
Cu	698	6	< 1%	$3{,}5 \times 10^7$
SnCu	674	7	< 2%	$3{,}4 \times 10^7$
SnCu	721	4	< 1%	$2{,}6 \times 10^7$
SnCu	792	5	< 1%	$3{,}9 \times 10^7$

TABLE 1: Bacteria counting results for Cu and SnCu electrodes after 40 minutes exposure

Cell counts results for each sample are presented in Table 1. They show that Dead on Alive ratio remain stable for both SnCu and Cu electrodes. Within this experimental setup, more than 98% of bacteria were

alive after contact with the electrodes. From these values and in such experimental conditions, we can confirm that both Cu and SnCu electrodes had no impact on bacterial membranes and thus no influence on our EIS measurements.

B. EIS results

Theoretical EIS simulations

A prior study proposed a one-layered bacterium membrane model instead of three in order to represent its dielectric properties $\sigma_{wall} = 3.7 \times 10^{-5}$ S m^{-1}, $\varepsilon r_{wall} = 13.5$ (Xavier *et al.*, 2017). Using this model, along with dielectric properties for the cytoplasm σ_{cyt}, εr_{cyt} and the external medium σ_{med}, εr_{med}, the frequency behaviour of a bacterium can be analysed. Dielectric values for cytoplasm $\sigma_{cyt} = 0.22$ S m^{-1} and $\varepsilon r_{cyt} = 40$ are taken from (Bai *et al.*, 2006; Asami *et al.*, 1980). For an Alive bacterium suspended in a weakly conductive medium such as DI water, the ions remaining after centrifugation will influence the overall medium conductivity. This contribution has been estimated to $n = 1.6 \times 10^7$ charges per bacterium. It includes charges coming from the Gouy-Chapman-Stern electric double-layer model (section I.C) and contributions from ions remaining after centrifugation as well as other ions than Na^+ and Cl^-. To link this value to conductivity, we consider a simplified medium containing only Na^+ and Cl^- ions.

Our working hypothesis translates to a medium homogenisation ($\sigma_{cyt} = \sigma_{med}$) when a treatment is applied to a bacterium. Otherwise, the external medium is considered weakly conductive ($\sigma_{cyt} \gg \sigma_{med}$).

The choice has been made to simulate both an Alive bacterium and a Treated one. For both COMSOL® FEM simulations, choosing the volume size surrounding the bacterium allows to fix the bacterial concentration. For example, a simulation volume of $10 \times 10 \times 10\ \mu m^3$ corresponds to a 3×10^8 CFU mL^{-1} bacterial concentration. Within this simulation parameters, by using $\sigma = n\, e\, \mu_{Na^+} + \mu_{Cl^-}$, we obtain respectively a $\sigma_{med} = 3.5 \times 10^{-4}$ S m^{-1} conductivity value for the external medium for an Alive bacterium and $\sigma_{med} = 4.2 \times 10^{-4}$ S m^{-1} for a Treated one. Concerning εr_{med}, a value of 78 has been chosen, corresponding to DI water at ambient temperature. In order to model the whole bacterial solution, the total volume has been approximated as a finite sum of the aforementioned bacterium simulations, taken as unity values.

Figure 2 presents simulated impedance modulus and phase for frequencies between 500 Hz and 1 GHz.

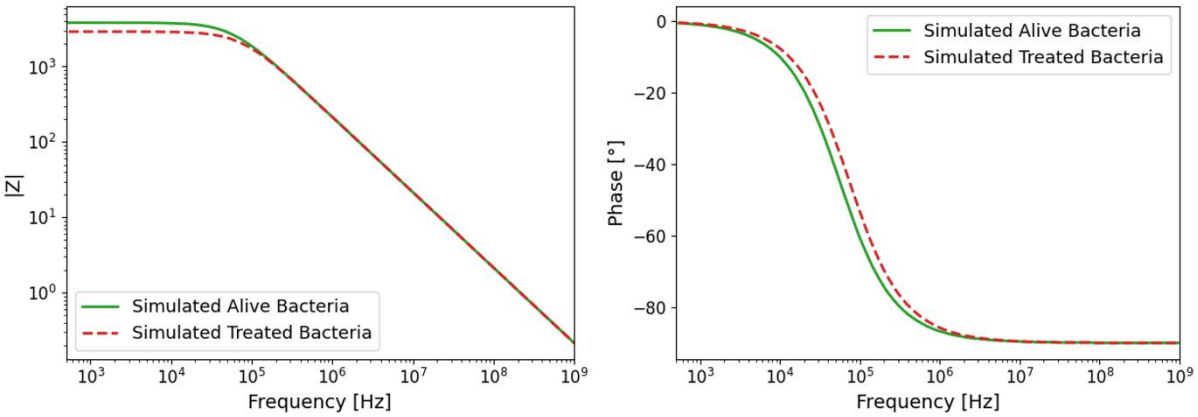


FIGURE 2. EIS simulation on *Escherichia Coli* model corresponding to 3×10^8 CFU mL^{-1} for Alive and Treated solutions.

These results show a behaviour following the typical response for a first-order lowpass RC circuit for both simulations: the impedance modulus Bode plot presents a plateau on the impedance modulus and a phase shift between 0° and -90° for frequencies under ~ 100 kHz, then a modulus decreasing with a linear slope, which validates our choice to model the system as a single equivalent RC circuit. The difference between Alive and Treated bacteria behaviours lies in the observed shift for both the plateau value and the cut-off frequency. Lower plateau and higher cut-off frequencies for the Treated case are consistent with a lower equivalent resistance resulting from a higher overall conductivity σ_{med} when compared to the Alive one. In these conditions, simulation results capture a single electrochemical phenomenon (resistive-capacitive effect), and no visible impact of the bacterial membranes can be observed in the simulated impedances, which means that the membrane polarization mechanisms are not directly accessible through a direct analysis from EIS data. Simulations without the bacterium for both σ_{med} conductivity conditions show no visible difference when compared to the ones taking the bacterial membrane into account, confirming this assumption. In conclusion, these results show that directly looking at conductivity variations only allows to get information about the overall aqueous medium electrical properties.

EIS experimental data: pollution detection in water

This section presents EIS experimental data for frequencies between 1 kHz and 20 MHz on *E. Coli* bacterial solution samples prepared as described in section I.E and I.G.

Preliminary measures with our experimental setup established a minimum bacterial concentration of 10^7 CFU mL^{-1} in order to guarantee overall solution conductivity variations over 10^{-5} S m^{-1}, otherwise biological variation is more important than the magnitude of the measure.

The following results are for 10^8 CFU mL^{-1}. Deviations from the model stem from the approximation of the cytoplasm as a purely ionic Na^+ / Cl^- solution without other elements. Additionally, experimental limitations regarding the washing efficiency could lead to a higher residual ionic strength in the medium than theoretically assumed.

EIS spectra for one population experiments at $t = 0$ are shown in Figure 3, separating Ampicillin and Ivermectin spectra on two distinct Bode plots. Box plots illustrate the variability across five measurements per sample.

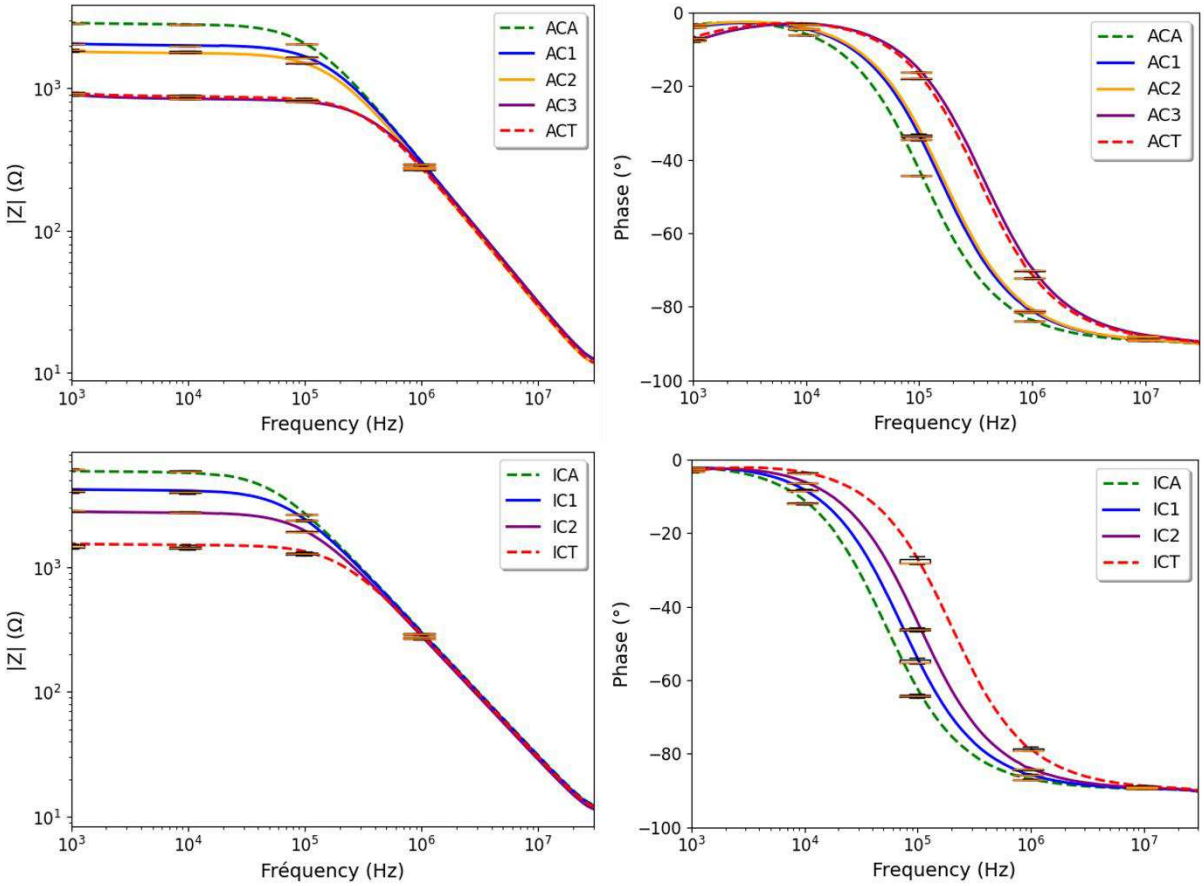


FIGURE 3. EIS spectra of *E. coli* controls (Alive: [**ACA**, **ICA**], Heat-Treated: [**ACT**, **ICT**]) and samples exposed to Ampicillin (10, 20, 100 µg mL^{-1}: [**AC1–AC3**]) and Ivermectin (15, 45 µg L^{-1}:[**IC1–IC2]**) at t = 0 min.

They all show the same first-order lowpass RC circuit behaviour seen in the simulations. This means that the measured response is mainly governed by resistive-capacitive processes in the aqueous solution.

Since R and C reflect the medium electrical properties for any given sample, we proceeded to extract these two values from all measured data. These results are synthesized in Figure 4: for each pollutant, graphs depicting the evolution of R and C for each tested condition (all populations) over time are shown. For each tested condition, the dots represent individual experimental values, the median value is represented by the horizontal line, and the solid box and bars indicate interquartile ranges (IQR) and 1,5×IQR respectively. This global representation allows to follow the changes in electrical properties for all samples tested under different conditions over time.

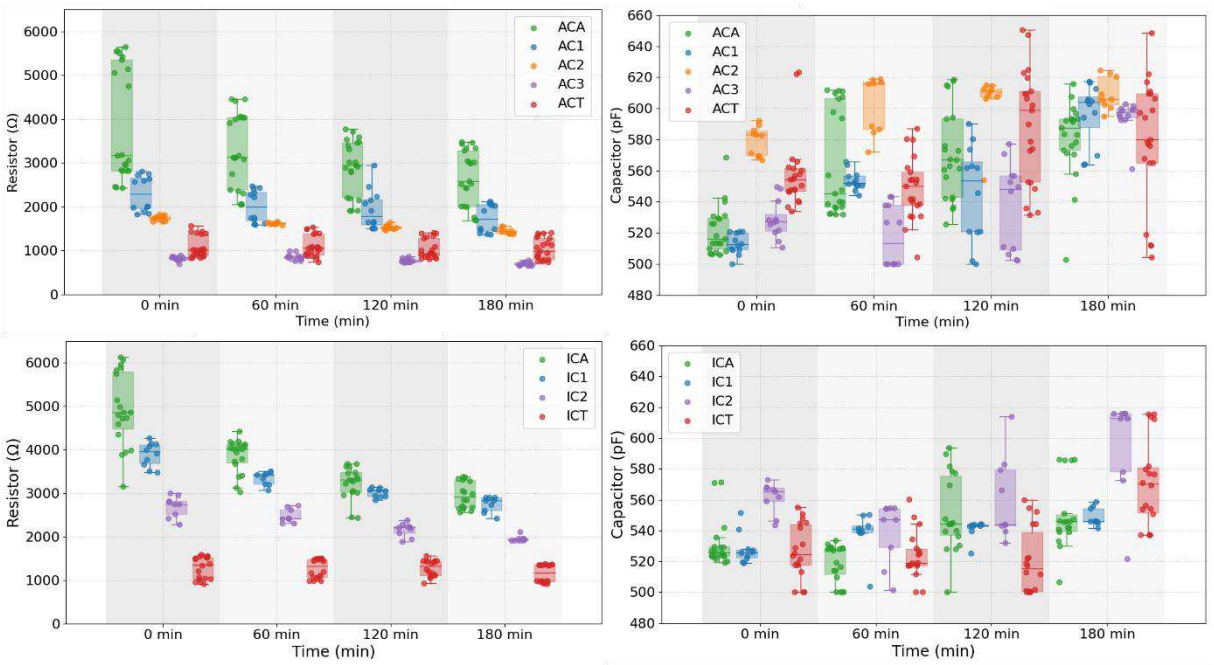


FIGURE 4. Variation of *R* and *C* parameters from all impedance spectra data for *E. Coli* controls (Alive: [**ACA**, **ICA**], Heat-Treated: [**ACT**, **ICT**]) and samples exposed to Ampicillin (10, 20, 100 µg mL^{-1} : [**AC1–AC3**]) and Ivermectin (15, 45 µg L^{-1} :[**IC1–IC2**]) over time (t = 0, 60, 120 and 180 min).

Alive control samples resistance values tend to progressively decrease over time (≈ 4.5 kΩ ± 1.5 kΩ for t = 0 vs. 2.5 kΩ ± 850 Ω for t = 180 min). This suggests an evolution of the physiological state of bacteria over time, which is consistent with Fick-governed ion diffusion from within and around the bacteria due to ionic concentration differences in the solution. Concerning Heat-Treated control samples, resistance values are stable in time and significantly inferior (1.5 kΩ ± 550 Ω), reflecting the absence of metabolism as well as a damaged membrane.

Polluted-sample-condition resistance values vary with concentration, but averages are consistently distinct from Alive controls and consistently lower for increasing concentrations. Resistance values at t = 0 are given to illustrate this point for Ampicillin (10 µg mL^{-1} = 2 010 $\Omega \pm 403\ \Omega$; 20 µg mL^{-1} = 1620 $\Omega \pm 23\ \Omega$; 100 µg mL^{-1} = 890 $\Omega \pm 84\ \Omega$) and Ivermectin (15 µg L^{-1} = 3 890 $\Omega \pm 230\ \Omega$; 45 µg L^{-1} = 2 700 $\Omega \pm 270\ \Omega$). These results point towards confirmation that electrical behaviour and cellular integrity are closely dependent. A particular case concerns the solutions exposed to a 100 µg mL^{-1} Ampicillin concentration, which show resistance values close to Heat-Treated control samples ($\Delta R_{Heat/100\mu g/mL}(T0) < 60\ \Omega \pm 60\ \Omega$) and stable in time.

This highest chosen concentration, 25 times higher than the measured MIC, shows therefore similar effects to a heat treatment on *E. Coli* from the point of view of overall solution conductivity variation. Resistance values for polluted samples decrease over time for both Ivermectin ($\Delta R_{T180-T0}$ 15 $\mu g\ L^{-1} = -546\ \Omega \pm 63\ \Omega$; 45 µg L^{-1} = $-221\ \Omega \pm 105\ \Omega$) and Ampicillin ($\Delta R_{T180-T0}$ 10 $\mu g\ mL^{-1} = -296\ \Omega \pm 49\ \Omega$; 20 µg mL^{-1} = $-112\ \Omega \pm 30\ \Omega$) except for the aforementioned highest concentration. The working hypothesis is that the stronger the effect of the chemical agent on the bacteria, the greater the observed ion release will be. For solutions containing Ampicillin, this is likely due to cell wall deterioration. Concerning Ivermectin, the results for the highest concentrations show that the ion concentration in the medium is increased. It may stem from membrane permeabilization and consequent ion leakage (Flores-Cosío *et al.*, 2020).

Concerning capacitances, all obtained values are within a small interval ($500 - 610$ pF), making it difficult to draw any conclusions concerning the variation of this parameter. For our current setup, the contribution of cell wall permittivity variation is not significant enough in the impedance measurement.

These results show the interest of the electrochemical approach in order to rapidly discriminate between alive and treated bacteria. Extraction of resistance values from EIS spectra allows efficient separation of bacterial populations since the initial contact to pollutants, while capacitance values do not allow to extract any additional information. Thus, using variation of sample medium conductivity brings an efficient but partial image of the bacteria physiological state. Still, it presents as a sufficient method for an early warning biosensor application approach, where a fast response is essential.

Finally, it should be noted that all observed variations on resistance are reproductible on all three replicates.

C. DRT analysis

DRT analyses presented in this section are performed using dearEIS free software (Yrjänä, 2022), the TR-NLLS method and a $\lambda = 10^{-4}$ parameter (Kulikovsky, 2020).

DRT on simulation results

Up to now, there is no scientific consensus for establishing a link between DRT peaks detected on a given τ region and a given electrochemical process (Ramírez-Chavarría *et al.*, 2021; Lee *et al.*, 2025). In order to get a clearer picture, we have performed a DRT analysis on the impedance data from Alive and Treated single bacterium simulations presented in the previous section, along with a simulation for the medium with no bacterium under the same conditions. These results are presented in Figure 5.

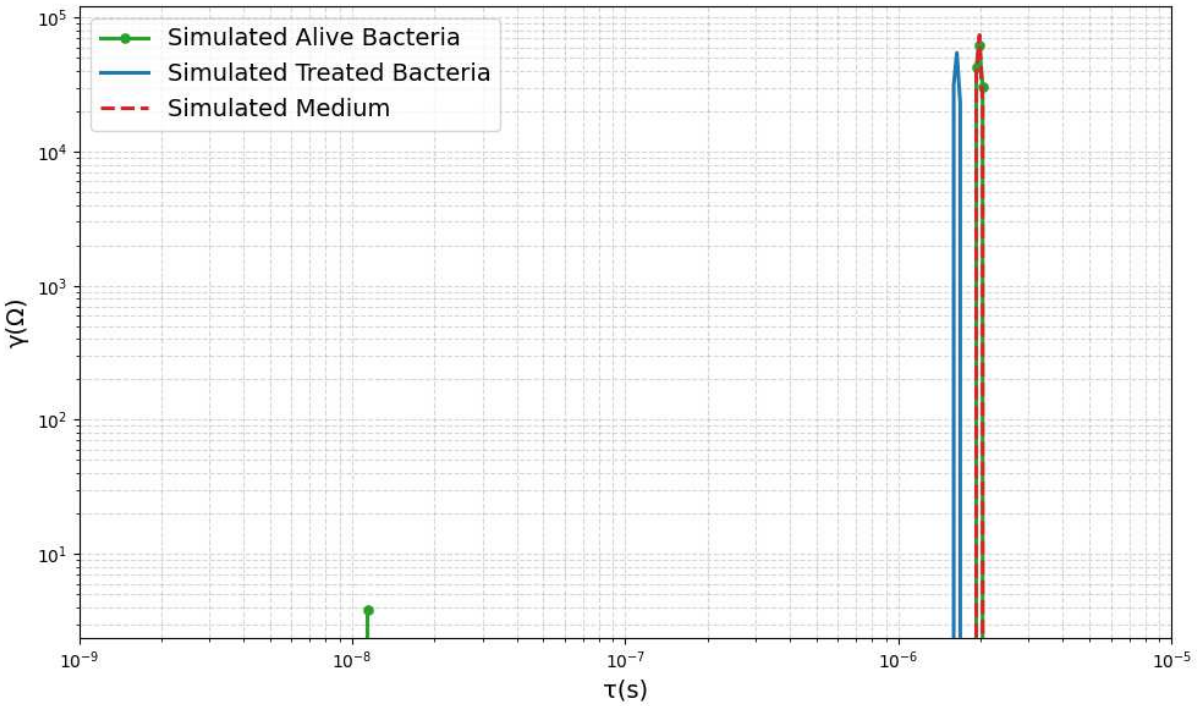


FIGURE 5. DRT spectrum computed from single Alive and Treated *E. coli* model corresponding to 1×10^9 CFU mL^{-1}, along with medium-only simulation.

The DRT for the medium-only simulation presents a single, high-amplitude peak, representing the resistive-capacitive process image for the homogeneous medium and giving its position in the DRT

spectrum around 2 µs. The results for the Alive single bacterium have a high-amplitude peak around the same position, plus a second, much smaller peak around 10^{-8} s. Finally, the Treated single bacterium has a single high-amplitude peak, also on the 10^{-6} s region, but whose position has slightly shifted towards a smaller τ value (~ -15% of the absolute value).

We can thus identify peaks within two distinct τ regions. Firstly, the $\tau_1 \sim 10^{-6}$ s region, containing all high-amplitude peaks from the three simulated cases, which is linked to the resistive-capacitive process, the main contribution in the electrochemical aqueous medium response. This affirmation is confirmed by calculating the *RC* values from extracted *R* and *C* experimental data for control solutions presented in the previous section, which range between 0.8 and 2.9 µs. The second region, $\tau_2 \sim 10^{-8} s$, only has one peak appearing for the Alive single bacterium simulation. We can thus infer that it translates a second electrochemical process, several orders of magnitude smaller in contribution, and only present in the alive bacterium simulation: namely, the bacterium.

By observing the τ_1 region, we can discriminate between Alive and Heat-Treated single bacterium simulations through the significant 0.4 µs decrease in the τ value (representing 15% in absolute value). This is a logical result, and consistent with experimental data for Alive and Heat-Treated solutions already discussed, since higher conductivity values have already been shown to translate into lower equivalent resistance values and consequently higher cut-off frequencies (and lower τ values). The peak amplitude also decreases (from 6.4×10^4 Ω to 5.4×10^4 Ω).

DRT on experimental data: pollution detection in water

A DRT analysis has been applied to all EIS experimental data for both Ampicillin and Ivermectin tests described in section II.B (including the Alive and Heat-Treated control samples), focusing on the τ_1 region, which as seen previously, contains the peak of the $\gamma(\tau)$ function associated to the relaxation times characteristic of resistive-capacitive behaviour in the aqueous medium, and which is the main contribution to the EIS experimental data. Figure 6 presents a 3D plot around the τ_1 region for each pollutant, showing the control solutions plus a selection of the tested concentrations over time for two representative populations in order to illustrate the evolutions.

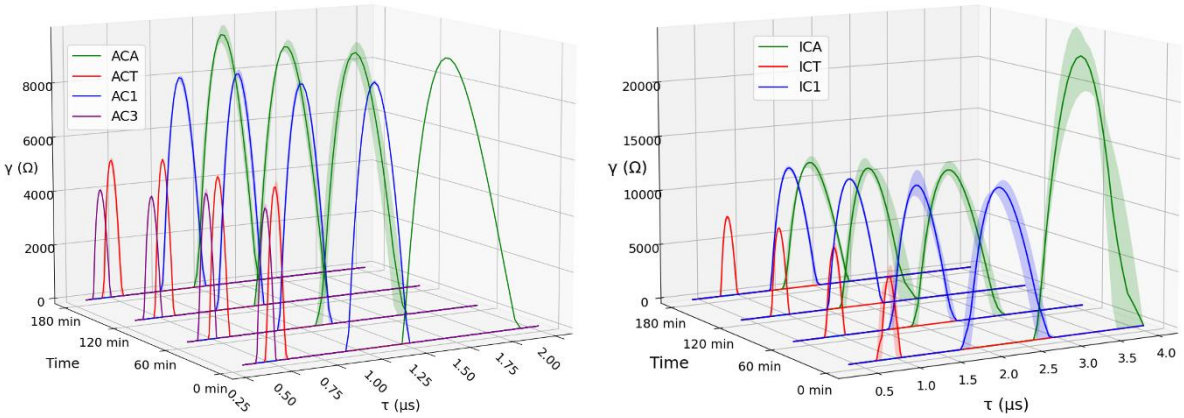


FIGURE 6. DRT spectra centred on the main RC peak (τ_1 region) for Ampicillin (left) and Ivermectin (right) experiments over time; Axes: $\gamma(\tau)$ (Ω), τ (µs), time (min); Left (Ampicillin) controls (Alive: [**ACA**], Heat-Treated: [**ACT**]) and samples exposed to Ampicillin (10, 100 µg mL^{-1} : [**AC1,AC3**]); Right (Ivermectin) controls (Alive: [**ICA**], Heat-Treated: [**ICT**]) and samples exposed to Ivermectin (15 µg L^{-1} : [**IC1**])

In the following analysis, all data from all tests are considered. For both Ampicillin and Ivermectin tests, DRT results present, as expected, a predominant peak in the τ_1 region ($\tau \approx 10^{-6}$ s) for all samples, with

τ values and markedly higher for Alive control solutions compared to the rest (Heat-Treated control and polluted solutions). This contrast between τ values allows to discriminate between treatments, from the moment bacterial solutions are put in contact with pollutants ($t = 0$). To illustrate this point, here are the Δτ values with the Heat-Treated control as reference for the Alive solutions: $|\Delta\tau_{Alive-Heat-treated}| = 2 \pm 0{,}9$ µs; the Ampicillin-treated solutions: $|\Delta\tau_{10\mu g/mL-Heat-treated}| = 0{,}9 \pm 0{,}3$ µs; $|\Delta\tau_{20\mu g/mL-Heat-treated}| = 0{,}7 \pm 0{,}1$ µs; $|\Delta\tau_{100\mu g/mL-Heat-treated}| = 0{,}1 \pm 0{,}06$ µs; and the Ivermectin-treated solutions: $|\Delta\tau_{15\mu g/L-Heat-treated}| = 1{,}20 \pm 0{,}4$ µs ; $|\Delta\tau_{45\mu g/L-Heat-treated}| = 0{,}9 \pm 0{,}2$ µs.

When studying the evolution over time for the Alive controls, a general decrease of τ values along with a slight decrease in the associated γ peak values can be observed for all peaks. This dynamic, representing a variation between 22% and 36% after 180 minutes depending on the populations, is consistent with a progressive medium conductivity increase, linked to ionic diffusion from bacteria to DI water. This was already observed in the extracted *RC* data from measured impedances as a decrease in *R*, consequently translated into a smaller τ.

Heat-Treated solutions remain stable over time, both in terms of τ and γ values, which are considerably smaller than alive controls. This is consistent with the absence of metabolism as well as membrane damage brought by the heat treatment.

Concerning the analysis for Ampicillin-treated solutions over time, a behaviour similar to the Heat-Treated control solutions is observed: their peaks remain stable over time. This can be illustrated by calculating the τ relative difference after 180 min exposure, $\Delta\tau_{t180-t0}$, which is 11.2 % for 10 µg mL^{-1}, 3.7 % for 20 µg mL^{-1} and 2.2 % for 100 µg mL^{-1} (For reference, 22 % for Alive and 4% for Heat Treated controls). These results are coherent with a fast bactericide effect for Ampicillin on the studied populations.

As for Ivermectin-treated solutions analysis over time, the behaviour dynamics is similar to the one observed for Alive controls, but changes are slower and smaller in magnitude. The τ relative difference $\Delta\tau_{t180-t0}$ varies between 27 % for 15 µg L^{-1} and 11 % for 45 µg L^{-1} (For reference, 36.5 % for Alive and 4 % for Heat-Treated controls). This is a more progressive response pointing towards different action mechanisms, leading to a much slower alteration of the ionic balance and the bacterial membrane.

In our conditions, we have established, both through simulations and experiments, that τ_1 is an image of the effective *RC* for the test medium, mainly driven by the conductivity of the solution. With increasing conductivity values, a subsequent decrease in the equivalent resistance *R* translates into an increase for the associated cut-off frequency and a decrease for the associated $\tau = 1/(2\pi f_c)$. Finally, the coherence between EIS results and DRT analysis confirms that τ_1 and its temporal evolution are fast and consistent markers in order to discriminate between controls (Alive and/or Heat-Treated) and polluted bacterial population samples. These experiments confirm the strong interest of this original approach using DRT for impedance analysis thanks to its fastness, easiness of implementation and reliability.

III. CONCLUSION

In this article, we have proposed a new impedancemetric biosensor using *E. coli* bacteria as bioreceptors. The sensing principle assumes that the ionic diffusion between the bacteria cytoplasm and its environment during an accidental pollution is modifying the membrane permeability of bacteria.
Presented results begin with a validation of the measurement setup that has proven that the Cu-based electrodes used are not toxic for the bacteria under our experimental conditions.
The sensor behaviour has then been modelled using a semi-analytical model along with FEM simulations. EIS responses of bacterial control solutions, (comprising Alive and Treated bacteria) have been simulated and show that directly looking at conductivity variations only allows to get information

about the overall aqueous medium electrical properties. This method was applied experimentally to detect the effect of the antibiotic Ampicillin and the anthelminthic Ivermectin on an *E. Coli* bacterial solution. Moreover, despite the scarcity of studies regarding its interaction with bacteria, Ivermectin induced comparable signal variations at concentrations orders of magnitude lower than Ampicillin (µg L^{-1} vs µg mL^{-1}), highlighting the critical environmental risk posed by this emerging pollutant.

Analysis of EIS spectra enables an immediate ($t = 0$) and effective discrimination of varying pollutant concentrations: Ampicillin (10, 20, and 100 µg mL^{-1}) and Ivermectin (15 and 45 µg L^{-1}). The obtained detection limits are highly relevant for environmental monitoring, as they are well below or close to reported aquatic thresholds of 5 mg mL^{-1} for Ampicillin (Samandari, 2022) and 5 µg L^{-1} for Ivermectin (Mesa, 2020).

These results confirm solution conductivity as a robust, dose-dependent variable for bacterial viability. Furthermore, literature has established that membrane permeabilization is a phenomenon distinct from cell inactivation (Virto et al., 2005). The kinetics of ion dispersion observed in this study are significantly faster and more intense than the natural background drift, confirming a specific stress response.

A complementary study of these data using Distribution of Relaxation Times (DRT) have also been conducted. Analysis of simulated data provides evidence of a link between DRT peaks detected on a given τ region and a given electrochemical process and illustrates the power of this tool. All experimental DRT spectra at $t = 0$ exhibit a dominant peak near $\tau \approx 10^{-6}$ s, with larger τ values for Alive controls and clear τ-shifts versus Heat-Treated for both Ampicillin and Ivermectin allowing us to discriminate.

Future work towards concrete applications includes taking into account the impact that conductive pollutants could have on measurements. Preliminary results (not yet published) seem to show that overall solution conductivity can be increased while retaining measurement discrimination.

Beyond impedance measurements, the novel application of the DRT algorithm to this bacterial biosensor paves the way for accurate and rapid discrimination of water toxicity, validating its potential as a real-time early-warning system for emerging pollutants such as Ivermectin.

CRediT authorship contribution statement

Declaration of competing interest

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References

Asami, K., Hanai, T., Koizumi, N., 1980. Dielectric analysis of Escherichia coli suspensions in the light of the theory of interfacial polarization. Biophys. J. 31(2), 215–228. https://doi.org/10.1016/S0006-3495(80)85052-1

Ashraf, S., Chaudhry, U., Raza, A., Ghosh, D., Zhao, X., 2018. In vitro activity of ivermectin against Staphylococcus aureus clinical isolates. Antimicrob. Resist. Infect. Control 7(1), 27. https://doi.org/10.1186/s13756-018-0314-4

Bai, W., Zhao, K., Asami, K., 2006. Dielectric properties of E. coli cell as simulated by the three-shell spheroidal model. Biophys. Chem. 122(2), 136–142. https://doi.org/10.1016/j.bpc.2006.03.004

Campanale, C., Massarelli, C., Losacco, D., Bisaccia, D., Triozzi, M., Uricchio., 2021 The monitoring of pesticides in water matrices and the analytical criticalities: *Chem.*144, 116423. https://doi.org/10.1016/j.trac.2021.116423

Cass, A., Finkelstein, A., Krespi, V., 1970. The ion permeability induced in thin lipid membranes by the polyene antibiotics nystatin and amphotericin B. J. Gen. Physiol. 56(1), 100–124. https://doi.org/10.1085/jgp.56.1.100

Chopra, S. L., Dale, D. G., & Blackwood, A. G.,1963 Effect of ampicillin on E. Coli of swine origin. *Canadian Journal of Comparative Medicine and Veterinary Science*: *27*(9), 223. No DOI

Ciucci, F., Chen, C., 2015. Analysis of electrochemical impedance spectroscopy data using the distribution of relaxation times: A Bayesian and hierarchical Bayesian approach. Electrochim. Acta 167, 439–454. https://doi.org/10.1016/j.electacta.2015.03.123

Deghelt M, Cho SH, Sun J, Govers SK, Janssens A, Dachsbeck AV, Remaut HK, Huang KC, Collet JF. Peptidoglycan-outer membrane attachment generates periplasmic pressure to prevent lysis in Gram-negative bacteria. Nat Microbiol. 2025 Aug;10(8):1963-1974. https://doi.org/10.1038/s41564-025-02058-9. Epub 2025 Jul 29. PMID: 40730909.

Flores-Cosío, G., Herrera-López, E.J., Arellano-Plaza, M., Gschaedler-Mathis, A., Kirchmayr, M., Amaya-Delgado, L., 2020. Application of dielectric spectroscopy to unravel the physiological state of microorganisms: Current state, prospects and limits. Appl. Microbiol. Biotechnol. 104(14), 6101–6113. https://doi.org/10.1007/s00253-020-10677-x

Hassani, S., Momtaz, S., Vakhshiteh, F., Maghsoudi, A.S., Ganjali, M.R., Norouzi, P., Abdollahi, M., 2017. Biosensors and their applications in detection of organophosphorus pesticides in the environment. Arch. Toxicol. 91(1), 109–130. https://doi.org/10.1007/s00204-016-1875-8

Heileman, K., Daoud, J., Tabrizian, M., 2013. Dielectric spectroscopy as a viable biosensing tool for cell and tissue characterization and analysis. Biosens. Bioelectron. 49, 348–359. https://doi.org/10.1016/j.bios.2013.04.017

International Organization for Standardization (ISO), 1995. Water quality — Pseudomonas putida growth inhibition test (Pseudomonas cell multiplication inhibition test). ISO 10712:1995. Geneva: ISO. No DOI

Jennifer Laurent, Mamadou Diop, Rachid Amara, Cédric Fisson, Jean Armengaud, Pierre Labadie, Hélène Budzinski, Jérôme Couteau, Géraldine Maillet, Stéphane Le Floch, Jean Laroche, Vianney Pichereau, 2024. Relevance of flounder caging and proteomics to explore the impact of a major industrial accident caused by fire on the Seine estuarine water quality. Marine Pollution Bulletin. Vol. 201, 116178. https://doi.org/10.1016/j.marpolbul.2024.116178.

Justino, C.I.L., Freitas, A.C., Pereira, R., Duarte, A.C., Rocha-Santos, T.A.P., 2015. Recent developments in recognition elements for chemical sensors and biosensors. TrAC, Trends Anal. Chem. 68, 2–17. https://doi.org/10.1016/j.trac.2015.03.006

Kulikovsky, A., 2020. PEM fuel cell distribution of relaxation times: a method for the calculation and behavior of an oxygen transport peak. Phys. Chem. Chem. Phys. 22, 19131–19138. https://doi.org/10.1039/D0CP02094J

Lambert, P.A., 2002. Cellular impermeability and uptake of biocides and antibiotics in Gram-positive bacteria and mycobacteria. J. Appl. Microbiol. 92(Suppl.), 46S–54S. https://doi.org/10.1046/j.1365-2672.92.5s1.7.x

Lee, K.-S., Yoo, K.-H., Lee, S.-M., 2025. Early detection of bacteria in the lag phase using the distribution of relaxation times analysis. Electrochim. Acta 524, 145973. https://doi.org/10.1016/j.electacta.2025.145973

Li, B., Logan, B.E., 2004. Bacterial adhesion to glass and metal-oxide surfaces. Colloids Surf. B Biointerfaces 36(2), 81–90. https://doi.org/10.1016/j.colsurfb.2004.05.006

Liu, J., Wan, T.H., Ciucci, F., 2020. A Bayesian view on the Hilbert transform and the Kramers–Kronig transform of electrochemical impedance data: Probabilistic estimates and quality scores. Electrochim. Acta 357, 136864. https://doi.org/10.1016/j.electacta.2020.136864

Macdonald, J. Ross, 1992. Impedance Spectroscopy. Annals of Biomedical Engineering 20: 289–305. https://doi.org/10.1007/BF02368532

Mauriz, E., Calle, A., Manclús, J.J., Montoya, A., Escuela, A.M., Sendra, J.R., Lechuga, L.M., 2006. Single and multi-analyte surface plasmon resonance assays for simultaneous detection of cholinesterase inhibiting pesticides. *Sens. Actuators B: Chem.* 118(1–2), 399–407. https://doi.org/10.1016/j.snb.2006.04.085

Mehrotra, P. (2016). Biosensors and their applications–A review. *Journal of oral biology and craniofacial research*, *6*(2), 153-159. https://doi.org/10.1016/j.jobcr.2015.12.002

Mesa, L., Gutiérrez, M. F., Montalto, L., Perez, V., & Lifschitz, A. (2020). Concentration and environmental fate of ivermectin in floodplain wetlands: an ecosystem approach. *Science of the Total Environment*, *706*, 135692. https://doi-org.sid2nomade-1.grenet.fr/10.1016/j.scitotenv.2019.135692

Poortinga, A. T.; Bos, R.; Norde, W.; Busscher, H. J. (2002). Electric double layer interactions in bacterial adhesion to surfaces. *Surface Science Reports*, 47(1), 1–32. https://doi.org/10.1016/s0167-5729(02)00032-8

Ramírez-Chavarría, R.G., Sánchez-Pérez, C., Romero-Ornelas, L., Ramón-Gallegos, E., 2021. Time-constant-domain spectroscopy: an impedance-based method for sensing biological cells in suspension. IEEE Sens. J. 21(1), 185–192. https://doi.org/10.1109/JSEN.2020.3014569

Samandari, M., Movahedian Attar, H., Ebrahimpour, K., Mohammadi, F., & Ghodsi, S. (2022). Measurement of ampicillin and penicillin G antibiotics in wastewater treatment plants during the COVID-19 pandemic: a case study in Isfahan. *Environmental Health Engineering and Management Journal*, *9*(3), 201-211. http://dx.doi.org/10.34172/EHEM.2022.21

Stern, O., 1924. Zur Theorie der elektrolytischen Doppelschicht. Z. Elektrochem. Angew. Phys. Chem. 30(21–22), 508–516. https://doi.org/10.1002/bbpc.192400182

Virto R, Mañas P, Alvarez I, Condon S, Raso J. Membrane damage and microbial inactivation by chlorine in the absence and presence of a chlorine-demanding substrate. Appl Environ Microbiol. 2005 Sep;71(9):5022-8. https://doi.org/10.1128/AEM.71.9.5022-5028.2005

Wan, T.H., Saccoccio, M., Chen, C., Ciucci, F., 2015. Influence of the discretization methods on the distribution of relaxation times deconvolution: Implementing radial basis functions with DRTtools. Electrochim. Acta 184, 483–499. https://doi.org/10.1016/j.electacta.2015.09.097

Wilson, W.W., Wade, M.M., Holman, S.C., Champlin, F.R., 2001. Status of methods for assessing bacterial cell surface charge properties based on zeta potential measurements. J. Microbiol. Methods 43(3), 153–164. https://doi.org/10.1016/S0167-7012(00)00224-4

Xavier, P., Rauly, D., Chamberod, E., Martins, J.M.F., 2017. Theoretical evidence of maximum intracellular currents versus frequency in an Escherichia coli cell submitted to AC voltage. Bioelectromagnetics 38(3), 213–219. https://doi.org/10.1002/bem.22033

Yrjänä, V., 2022. DearEIS – A GUI program for analyzing impedance spectra. J. Open Source Softw. 7(80), 4808. https://doi.org/10.21105/joss.04808

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