Electrical Response of Bacteria Cells in Water as Detection Mechanism

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Abstract: The detection of bacteria in drinking water is considered a critical step for the treatment and quality analysis of water. *Escherichia coli* and *Pseudomonas aeruginosa* are among the most common bacteria found in water sources in different regions of Saudi Arabia. The detection is determined by using common biochemical tests and sophisticated machine analysis. These methods are not sustainable for continuous daily detection due to their cost and time consumption. However, the electric characterization and separation of bacteria is a cost-effective and promising approach due to its real-time diagnosis and integration capabilities. This study aims to identify the electric characterization of three strands of bacteria, *Escherichia coli*, *Bacillus cereus*, and *Pseudomonas aeruginosa*, prior to detection. The bacteria are subjected to electric fields with different amplitudes and frequencies on the top of microelectrodes. The movement and resistance of *Escherichia coli*, *Bacillus cereus*, and *Pseudomonas aeruginosa* cells are investigated before and after applying the electric field. The movement and reaction of bacteria are observed under a microscope where a stronger response is noticed at higher signal amplitude. Before applying the electric field, the resistances of the above bacteria are found to be 1.28, 11.85, and 7 MΩ. Then, these values change to 4.6, 19, and 3.65 MΩ, respectively, after applying the electric field. In addition, the separation of bacteria in a mixture of *Escherichia coli* and *Bacillus cereus* at different frequencies is also investigated and presented in this paper.

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1 INTRODUCTION

Water is considered to be a source of life. This statement reflects our needs and the importance of water in our daily live. The availability of clean drinking water is becoming a priority(Okafor et al., 2024). The challenge occurs in the presence of Bacteria, which are prokaryotic creatures found everywhere. They can live in several environments and regions, adapting to the surrounding area to survive and multiply (Alawi et al., 2024). Bacteria pose critical health issues when consumed in water or any contaminated environment that can allow the bacteria to grow in the human body, generating immune responses and harmful infection (Alqahtani et al., 2015). The detection of Bacteria in drinking water is considered a crucial factor for developing a clean environmental ecosystem that could enhance the quality of drinking water for society. Several studies in different regions of Saudi Arabia investigated the presence of several types of bacteria in water samples collected from sources such as water taps, roof tanks and tankers (Abada et al., 2019; Abdalwahab et al., 2017; Alaidarous et al., 2017). The types and concentrations of bacteria found in these studies were different according to the city and source from which they were collected. The detection results showed that *Pseudomonas aeruginosa* is the most common bacteria that was present in the samples collected from around Saudi Arabia cities (Abdalwahab et al., 2017). The second most common appearance was *Escherichia coli* (Tenaillon et al., 2010). This analysis helps to identify the bacteria that are commonly found in drinking water that could help in the development of more clean water for consumers. It is important to mention that each study represents many different types of bacteria, and the *Pseudomonas aeruginosa* and *Escherichia coli* mentioned above were the most mentioned in all studies (Alshammari et al., 2016).

The bacteria detection techniques used in these studies were the conventional bacteria detection methods. Polymerase Chain Reaction (PCR) tech-

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nique and genomic DNA amplification were used in the detection process by special devices such as applied biosystems prism 3730xl DNA analyzer (Abada et al., 2019). The main part of the DNA strand of the bacteria to be identified is 16S rRNA, which can be identified in several other techniques such as using GeneJET genomic DNA purification kit (Eid et al., 2017). There are other methods used in the detection of bacteria, which require sample preparation to enable the bacteria to grow, then using simple processes such as the seven biochemical tests to identify the bacteria species (Eid et al., 2017) (Al-Turk and Diab, 2009). Gram stain, named for Hans Christian Gram, is a technique that uses differential staining with a crystal violet-iodine complex and a safranin counterstain to distinguish between different strands of bacteria. Gram-positive and Gram-negative (Bartholomew and Mittwer, 1952). Gram-positive has one Thick peptidoglycan layer. Whereas Gram-negative has two thin peptidoglycan layers (Coico, 2006). These detection methods require sophisticated machines with special software and statistical packages to complete the detection of a single sample (Omer et al., 2014). Further, the complexity of some of these methods, besides the time and resources they require, make it necessary to find better approaches to detect bacteria in drinking water in real-time.

Although the process of detecting bacteria in water is complex and needs a lot of steps to completed, electric characterization is a preferable method for the detection of the specific type of bacteria in drinking water. The electric characterization always depends on conductivity and the permittivity of the bacteria (Rahim et al., 2018). These characteristics could vary between the different types of bacteria in terms of the shape and size of the bacteria to be detected (Chen et al., 2024; Ware et al., 2024). Identifying these bacteria characteristics using electronics can be a useful method to detect the different types of bacteria in real-time applications without the need to take any samples out from the sources and conduct several tests in the lab. This method will save a lot of resources and time in detecting bacteria in drinking water. An example of electric characterizations is the usage of the dielectrophoresis method to manipulate bacteria(Weber et al., 2021). Dielectrophoresis is the motion of polarized bioparticles, such as bacteria, in a liquid medium that has different permittivity and conductivity(Qian et al., 2014). This research is developing a new real-time mechanism for the detection of bacteria in drinking water. The system separates different types of bacteria by their electrical conductivity and permittivity. This new technique solves the problem of the regular wet lab test that takes one sample from the water, then separates the different types of bacteria, and uses a sophisticated machine to know the strand found in the sample, which is time-consuming. The nutrient agar and the bacteria media were prepared in the lab as a reference method to confirm the presence of bacteria cells. The electrical response and resistance of three bacteria strands were measured and investigated. Also, two types of bacteria were mixed and visualized under the microscope, and their reaction to the different frequencies was studied. The scope of the study is to capture the change in movement and behavior when bacteria cells pass in electric fields generated by microelectrodes. The rest of the paper is structured as follows. The following sections explain the methodology and materials used in this project. Section three presents the results obtained and provides a detailed discussion. The paper is concluded in section four.

2 MATERIALS AND METHODS

The experimental work is divided into sample preparation and bacteria manipulation. The sample preparation is started by preparing nutrient agar solution, where 28 grams of dehydrated powder (lab-prepared media) is added to 1000 milliliters of distilled water and mixed in a flask. The suspension is then heated to boiling to dissolve the medium completely. The dissolved medium is then autoclaved at 15 lbs pressure (121°C) for 15 minutes. Once the autoclave process is complete, the flask is taken out and cooled to a temperature of about 40-45°C. The media is then poured into sterile Petriove plates under sterile conditions. Once the media solidifies, the plates are placed at room temperature for a few minutes to remove any moisture present on the plates before use. Then, different bacteria samples are added to different watch plates (Abdalwahab et al., 2017). The bacteria strands were put in the agar solution, allowing it to grow and replicate for one day. Then, the samples are collected from the petri dish and centrifuged for 3 minutes with 5000 spin. After that, the samples were transferred to a new container with 1 ml of sterilized water (Topić Popović et al., 2023). The manipulation of bacteria started by taking a drop of the bacteria sample and placed on the electrodes. The electrodes are ITO on a glass substrate with a width and electrode gap of 100 and 50 μm, respectively. The electrodes are connected to a function generator to supply an AC signal with controlled amplitude (1, 5, and 10 V) and frequencies (0.6, 0.8, 1 MHz). The electrodes were placed under a microscope (ZIESS AX10) and connected to a com-

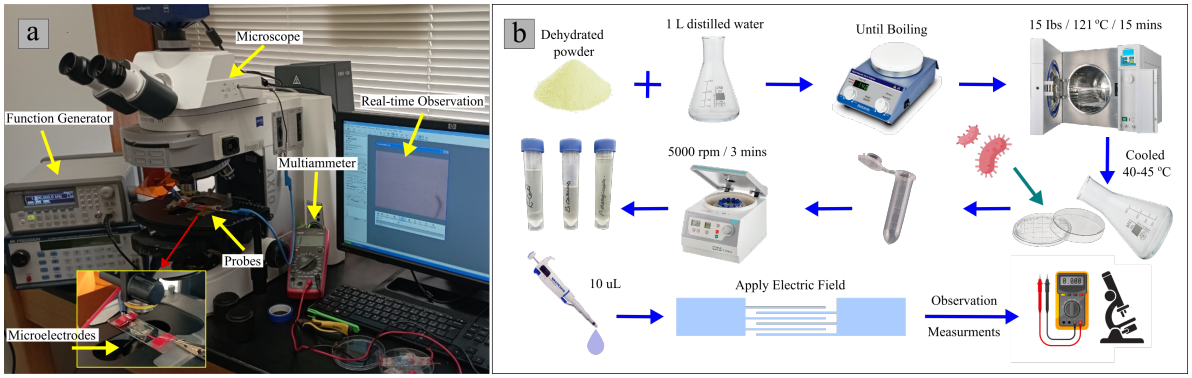

Figure 1: The methodology used in this work. (a) Experimental setup, (b) Steps of sample preparation and electrical characterization.

puter to visualize the real-time movement by ZEN Microscopy Software. The above-detailed methods are applied to *Escherichia coli*, *Bacillus cereus*, and *Pseudomonas aeruginosa*. The movement of bacteria at different electric fields is recorded and compared with their behaviour at no electric field. Further, the resistance of the bacteria sample is measured before and after applying the electric field using a multiameter.

3 RESULTS AND DISCUSSION

This section first discusses the behavior of a single type of bacteria under an electric field with different intensities. Then, the change in the bacteria resistance before and after applying the electric field is recorded and discussed in sub-section 3.2. Finally, sub-section 3.1 addresses the behavior of multiple bacteria types under different frequencies. The bacteria *Escherichia coli*, *Bacillus cereus*, and *Pseudomonas aeruginosa* were visualized and monitored under the microscope.

3.1 Single Bacteria Manipulation

Figure 2 shows the behavior of *Escherichia coli* at different electric field intensities and a fixed frequency of 1 MHz. First, as a reference, Figure 2a shows the distribution of *Escherichia coli* cells before applying the electric field. The bacteria started to move toward the electrode gap at a relatively slow speed when the electric field turned on using an AC signal with an amplitude of 1 V, as shown in Figure 2b. The bacteria motion became faster when the voltage increased to 5 and 10 V, as shown in Figure 2c and d. It is worth mentioning that this motion is a combination of vertical and horizontal motion as some bacteria cells were attracted from different heights above the electrodes, which explains the increase in the cell number compared to the number of cells at 0 V. A shaking movement in all directions of *Escherichia coli* was also detected as the voltage increased.

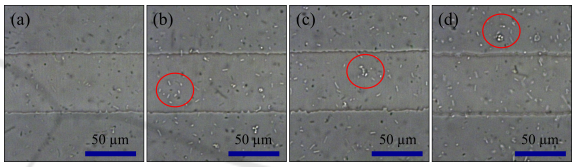

Figure 2: The electric response of *Escherichia coli*. (a) No signal applied, (b) 1 V, (c) 5 V, and (d) 10 V AC signal applied across the electrodes with fixed frequency of 1 MHz.

Figure 3a shows the behavior of *Bacillus cereus* before applying the electric field. At a signal of 1 V and 1 MHz, *Bacillus cereus* started to trap within the gap and above the electrode surface, as shown in Figure 3b. *Bacillus cereus* showed the most rapid movement in all strands, which was relatively higher than *Escherichia coli* and *Pseudomonas aeruginosa*. The number of trapped *Bacillus cereus* cells (see the green boxes) increases as the voltage increases, as shown in Figure 3c and d. The shivering movement in *Bacillus cereus* was less than it was in *Escherichia coli* as the speed difference indicates its active state.

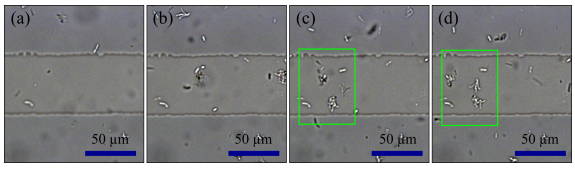

Figure 3: The electric response of *Bacillus cereus*. (a) No signal applied, (b) 1 V, (c) 5 V, and (d) 10 V AC signal applied across the electrodes with fixed frequency of 1 MHz.

Figure 4a shows a reference picture of *Pseudomonas aeruginosa* before applying the electric field. No changes were observed when applying an electric field of 1 V and 1 MHz, as shown in Figure 4b. A small number of bacteria cells (see the yellow

ovals) were observed at the electrode gap when the signal amplitude increased to 5 and 10 V, as shown in Figure 4c and d. In general, *Pseudomonas aeruginosa* was relatively slow compared to *Bacillus cereus*. The shivering movement in *Pseudomonas aeruginosa* was similar to that in *Escherichia coli*, indicating they share similar characteristics and behavior compared to *Bacillus cereus*.

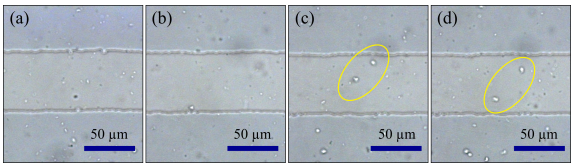

Figure 4: The electric response of *Pseudomonas aeruginosa*. (a) No signal applied, (b) 1 V, (c) 5 V, and (d) 10 V AC signal applied across the electrodes with fixed frequency of 1 MHz.

In conclusion, the difference in the behavior of the bacteria under electric fields is due to their cellular structure, which is confirmed using gram-positive and gram-negative tests. *Pseudomonas aeruginosa* is gram-negative with two thin peptidoglycan layer bacteria with rod-shaped structure (Diggle and Whiteley, 2020). *Escherichia coli* has almost similar size and length, and it is also gram-negative, explaining the similar behavior of these two bacteria (Tenaillon et al., 2010). On the other hand, *Bacillus cereus* is a gram-positive bacteria with a rod-shaped structure that varies in length from 3.0 - 5.0 µm. *Bacillus cereus* is bigger in size than *Escherichia coli* and *Pseudomonas aeruginosa* and has one thick peptidoglycan layer, explaining their strong response to the electric field (Logan and Vos, 2015).

3.2 Resistance Measurements

Measuring the resistance of the bacteria samples before and after applying the electric field was performed using a digital multimeter by connecting its probes with the electrode pads. This gives an indication of what happened during the application of the electric field. Figure 5 represents the average resistance of the bacteria samples before (black curve) and after (red curve) applying the electric field. The measurements were repeated 3 times after applying an AC signal with amplitudes of 10 V and frequency of 1 MHz applied for 1-2 minutes at similar conditions in the same electrode slide. In general, the difference in the resistance measurements in the bacteria samples before applying the electric field could be due to the size of the bacteria, the type in which it is gram-positive or gram-negative, the bacteria diameter and shape, and the conductivity of the medium. *Bacillus cereus* showed the highest resistance because of its larger size compared to *Escherichia coli* and *Pseudomonas aeruginosa*. Further, *Bacillus cereus* is considered to be a gram-positive bacteria with a thicker peptidoglycan layer, which explains the increase in the resistance of the bacteria. Both *Escherichia coli* and *Pseudomonas aeruginosa* have lower resistance values than *Bacillus cereus* as they are both gram-negative bacteria. *Pseudomonas aeruginosa* is the smallest strand in size, which is why it has the lowest resistance values. The change in the resistance after applying the electric field is because bacteria cells moved toward the electrodes and obstructed the current flow across the electrodes.

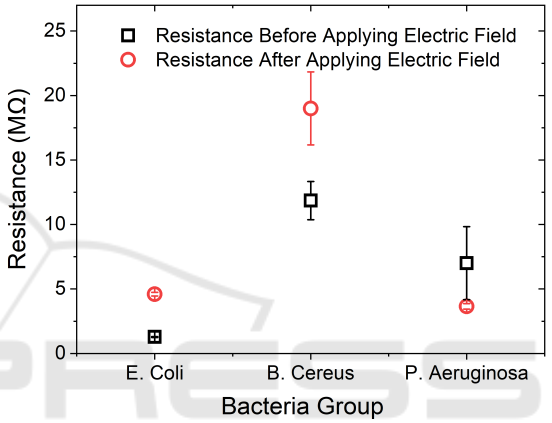

Figure 5: The resistance measurements of the studied bacteria before and after applying the electric field of 10 V and frequency of 1 MHz.

3.3 Multi Bacteria Manipulation

Escherichia coli and *Bacillus cereus* were mixed in the same medium with a volume of 5 µl each. The bacteria were observed under the microscope at different frequencies. It can be noticed that the bacteria cells have a rod shape with significant differences in their size. Figure 6a shows the case before applying the electric field where a semi-uniform distribution of the bacteria cell within the medium. In Figure 6b, the electric field was applied at a frequency of 1 MHz to the sample, and the change occurred as a shivering movement of both bacteria when they were in the electrode path. After some time, the bacteria started to become stable again before switching off the electric field. As the frequency decreased to 800 kHz, the bacteria showed stronger movement and shivering, as shown in Figure 6c. Also, it can be noticed here that the upper and middle parts of the electrode became darker, which indicates that a small number of E. Coli bacteria were trapped on the electrode surface. At 600

kHz, more E.coli bacteria are trapped on the electrode surface, as shown in Figure 6d. The dark area (as shown in the red box) indicated more cell trapping as a function of time and frequency. It can noticed from the shape of the bacteria that the trapped cells are E.coli, and what remained in the medium is *Bacillus cereus*. In conclusion, the isolation of E. Coli, trapping, and shivering of bacteria on the electrodes increases with the decrease in frequency.

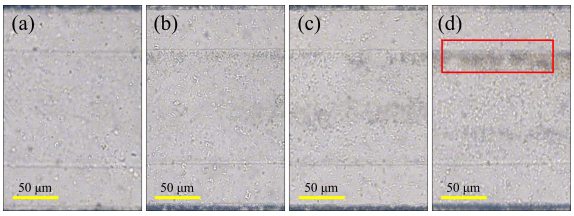

Figure 6: The electric response of a mixture of *Escherichia coli* and *Pseudomonas aeruginos*. (a) No signal applied, (b) 1 MHz, (c) 0.8 MHz, and (d) 0.6 MHz with fixed 10 V AC signal applied across the microelectrodes.

4 CONCLUSIONS

The detection of bacteria in drinking water is considered a critical need to prevent widespread bacterial infection. Recent studies showed that bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa* are present in drinking water sources at high percentages in several regions in Saudi Arabia. So far, the detection of these bacteria has been investigated using common biochemical tests and machine analysis, which is costly and requires a long time for the result. In this study, we investigated the electrical response of three different bacteria as a sensing mechanism. The bacteria's electric characteristics are affected by their length, diameter, and cell wall type. Through visualization of the bacteria under a microscope, the movement of bacteria at different voltages and frequencies was investigated. The difference in the behavior of the bacteria under electric fields was due to their cellular structure, which is confirmed using gram-positive and gram-negative tests. The electrical characterization in terms of the resistance was measured before and after the application of electric fields for all bacteria samples in this study.

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