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Analysis of Bacterial Characteristics Using the Electrical Impedance Spectroscopy Method

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Abstract: Microorganisms have various shapes, structures, and characteristics. This study uses the method of electrical impedance spectroscopy aimed at identifying and comparing the characteristics of *Escherichia Coli*, *Salmonella Typhi*, *and Staphylococcus Aureus.* Measurements from 1 Hz to 100,000 Hz show that *Salmonella Typhi* has the highest impedance value at low frequencies. In contrast, *Escherichia Coli* impedance decreases consistently, and *Staphylococcus Aureus decreases sharply* after 10 Hz. Significant changes are observed in the mid-frequency range of 100 Hz to 1000 Hz, with *Salmonella Typhi* showing the highest impedance values at 100 Hz compared to *Staphylococcus Aureus* and *Escherichia Coli*. At 100 Hz, *Salmonella Typhi* has the highest impedance value with a mass of 0,06 grams at approximately 39.000 Ohms, 0,08 grams at 35.000 Ohm, and 10 grams at 34.000 Ohm. This is followed by *Staphylococcus Aureus*, with a mass 0f 0,06 grams having an impedance value of 23.000 Ohms, 0,08 grams having a high impedance value of 31.000 Ohm, and 0,10 grams having an impedance value of 15.000 Ohm. *Escherichia Coli*, with a mass of 0.06 grams, has an impedance value of 9.000 Ohms, 0,08 grams with an impedance value of 5.000 Ohms, and 0,10 grams has an impedance value of 5.000 Ohms. Electrical Impedance Spectroscopy is effective for identifying and comparing *Escherichia coli*, *Staphylococcus aureu*, and *Salmonella typhi* as the intrinsic characteristics of bacterial cells more influence impedance than bacterial mass.

Keywords: *Escherichia coli*; Frequency; Impedance Spectroscopy; *Salmonella typhi*; *Staphylococcus aureus*.

Introduction

Microorganisms found in nature exhibit a variety of unique and distinctive shapes, structures, and characteristics, including bacteria [1]. Bacteria are microscopic organisms that are typically single-celled and lack a nuclear membrane. Generally, bacteria have a cell wall but do not contain chlorophyll. Despite their small size, bacteria play a crucial role in daily life, ranging from health to industry and the environment [2]. Bacteria are divided into two groups: Gram-positive bacteria and Gram-negative bacteria. Gram-negative bacteria have a thin *peptidoglycan* layer and an outer membrane containing *lipopolysaccharides*. These *lipopolysaccharides* are further classified into two types based on their structure: rough and smooth *lipopolysaccharides*. In contrast, Gram-positive bacteria have a thick *peptidoglycan* layer but lack an outer membrane [3].

Some bacteria are beneficial in the food industry, but some are harmful, such as those that cause food spoilage and diseases and infections in the human body. Three types of bacteria commonly found and responsible for various diseases in the human body are *Salmonella Typhi*, *Escherichia Coli*, and *Staphylococcus Aureus. Salmonella Typhi* is a bacterium that causes typhoid fever, which remains a significant health problem in many developing countries, particularly Indonesia [4]–[6]. *Escherichia Coli* is a Gram-negative bacterium found in the human intestines; most *Escherichia Coli* strains are harmless, but some can cause serious infections such as diarrhoea [7], [8]. Although *Escherichia Coli* and *Salmonella Typhi* share the same Gram-negative classification, they differ. *Escherichia Coli* falls into the category of smooth *lipopolysaccharides*, which have long and diverse O-chains. Smooth *lipopolysaccharides* protect the complement system and antibiotics, making these bacteria more resistant to environmental pressures.

In contrast, *Salmonella Typhi* falls into the category of rough *lipopolysaccharides*, which do not have long and diverse O-chains. This makes *Salmonella Typhi* more susceptible to the complement system and antibiotics due to the lack of protection provided by O-chains [9]. *Staphylococcus Aureus* is a Gram-positive bacterium that can cause various diseases, from minor skin issues such as itching from insect bites to more severe conditions like pneumonia [10].

To determine the total number of bacteria, the Total Plate Count (TPC) method and microscopy are commonly used, and they have a significant impact and contribution to microbiology. The Total Plate Count (TPC) method and microscopy require a longer processing time and are highly susceptible to contamination [3], [11]. However, more advanced methods have been needed to address various challenges. In this regard, electrical impedance spectroscopy is an analytical technique that measures the electrical response of a material to changes in applied electrical frequency. Electrical impedance spectroscopy can provide information about the quality of biological materials, as there is a relationship between the electrical properties of materials and their physiological conditions [12], [13].

The impedance value of biological tissues is influenced by frequency. The higher the frequency applied,

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the lower the impedance value. Changes in impedance values can provide information about membrane structure and intercellular fluids in biological tissues. At low frequencies, current still flows around the cells but begins to affect the space within the cell membrane. At high frequencies, current affects the entire intracellular space [14].

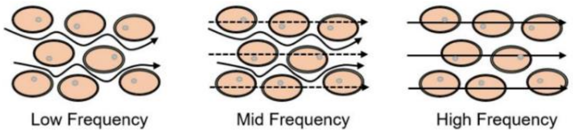

Figure 1. Cell current depends on frequency [14]

This study aims to identify and compare the characteristics of Escherichia coli, Staphylococcus aureus, and Salmonella typhi using electrical impedance spectroscopy, which can eventually be further developed for bacterial infection detection and treatment methods. The urgency of this research is based on the high incidence of bacterial infections that threaten global public health, where conventional detection methods are often slow and require specific laboratory conditions. Electrical impedance spectroscopy is a non-invasive, promising method that significantly reduces the time requneeded data collection. Furthermore, this method provides real-time results, allowing for early detection and faster, more accurate clinical decision-making. By implementing this method, healthcare systems can more efficiently manage bacterial infections, reduce the burden of care, and prevent further spread of infectious diseases by enhancing treatment effectiveness [15].

Research Methods

The research method begins with the preparation of tools and materials, including the use of masks and 70% alcohol to ensure cleanliness and prevent cross-contamination during the study. The bioimpedance spectroscopy equipment and electrodes are employed to measure the samples' electrical samples and impedance values. Bioimpedance Spectroscopy is the primary method because it provides non-invasive and efficient impedance data. The study uses three types of bacteria: *Escherichia coli* with a concentration of 1,8 x 10^7 CFU, *Salmonella typhi* with a concentration of 5,9 x 10^7 CFU and *Staphylococcus, aureus* with the same concentration of 2,35 x 10^9 CFU.

The cultured Nutrient Agar (NA) was chosen for its ability to provide necessary nutrients for bacterial growth and allow clear observation of colony formation. Bacterial cultures are prepared in sterile petri dishes and incubated at 37°C for 24-48 hours, according to the optimal growth conditions for each bacterium. After incubation, bacterial colonies are harvested from the culture media and weighed using a precise digital scale, resulting in masses of 0.06 grams, 0.08 grams, and 0.10 grams for each bacterium. The bacteria are then placed onto electrodes for bioimpedance spectroscopy measurement. The measurement frequency is set within the range of 1Hz-100kHz to capture impedance variations across different frequencies, with a current of 10μA. This broad frequency range allows for a comprehensive assessment of bacterial impedance responses under various conditions. Measurements are conducted repeatedly to ensure accuracy and to identify consistent patterns and trends. This method allows for the analysis of impedance data to determine the specific electrical characteristics of each bacterium and to compare the impedance responses between Escherichia coli, Salmonella typhi and Staphylococc,us aureus.

Figure 2. Colonies of *Escherichia Coli*, *Salmonella Typhi*, and *Staphylococcus Aureus* on Nutrient Agar (NA) Media

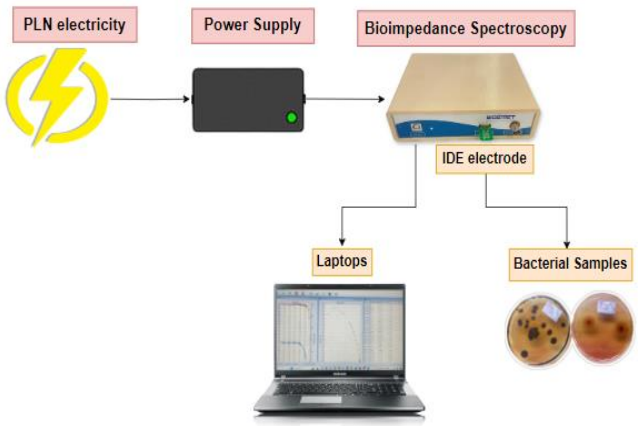

Figure 3. Electrical Impedance Spectroscopy Measurement with Bacterial Samples.

Based on Figure 3, the data collection process using bioimpedance spectroscopy begins with the provision of electrical from the main power grid, which is then stabilized by a power supply. The stabilized electricity is channelled to the bioimpedance spectroscopy device, connecting it to the IDE electrodes. Bacterial samples are placed on the electrodes at this stage to measure their electrical impedance response. Electrical impedance is a measure that combines resistance and reactance to the flow of electric current with the bacterial samples. The measurement data from the bioimpedance spectroscopy device is then transmitted to a computer via a type B USB cable. The computer displays the measurement results in graphs, ready for further analysis, allowing researchers to understand the electrical characteristics of the bacterial samples.

Results and Discussion

The study utilizes Electrical impedance spectroscopy (EIS) to classify three types of bacteria: *Escherichia coli*, *Staphylococcus aureus, and Salmonella typhi*. By measuring the electrical impedance response at various frequencies, each type of bacteria exhibits unique impedance characteristics. These measurements clearly depict the distinct electrical properties of the three bacterial species. The following impedance graphs illustrate the characteristics of each bacterium.

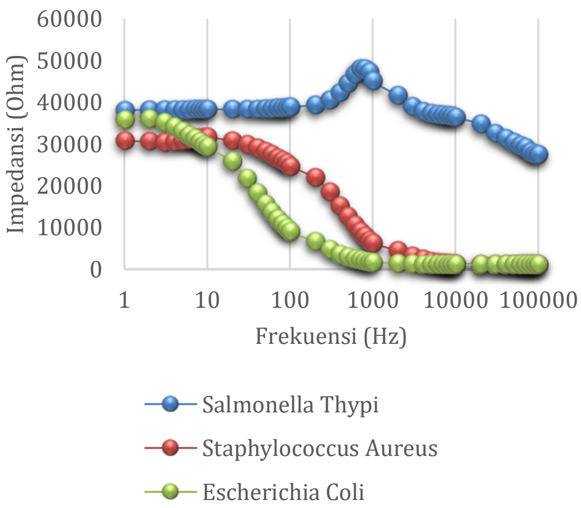

Figure 4. Electric Impedance Graph of Bacteria

Impedance is the total resistance in an electrical circuit when subjected to alternating current (AC). it combines the effects of resistance and reactance, which describe how the circuit responds to changes in current and voltage with varying frequencies. As the frequency increases, the impedance decreases, whereas a decrease in frequency results in an increase in impedance. Therefore, impedance is inversely proportional to frequency [16]. In Figure 4, the electrical impedance graph of Salmonella Typhi shows that the initial impedance at low frequencies 1 Hz – 10 Hz is around 40.000 Ohms. The graph shows slight fluctuations but remains relatively stable in 1 Hz – 100 Hz. At 1000 Hz, there is a sharp increase to approximately 50.000 Ohms, followed by a decrease at higher frequencies around 10.000 Hz. For *Staphylococcus Aureus,* the initial impedance is around 30.000 Ohms. This graph shows a more drastic decrease than *Salmonella Typhi*, especially after 10 Hz. There are fluctuations around 1000 Hz. At high frequencies of 10.000 Hz, the graph shows a further decrease and tends to level off around 10.000 Hz – 100.0000 Hz. Meanwhile, the graph for *Escherichia Coli* shows an initial impedance at low frequencies 1 Hz – 100 Hz of around 35.000 Ohm. The Impedance of *Escherichia Coli* decreases significantly compared to the other two bacteria, especially after 10 Hz. This graph shows a consistent decrease across the entire frequency range, reaching around 5.000 Ohm at high frequencies. The change in impedance with increasing frequency is due to the cell membrane functioning like a capacitor. The cell membrane behaves similarly to a capacitor because it can store and release electrical current, resulting in high impedance. However, electrical current can pass through the cell membrane at high frequencies more easily, leading to a decrease in impedance [17], [18] [19].

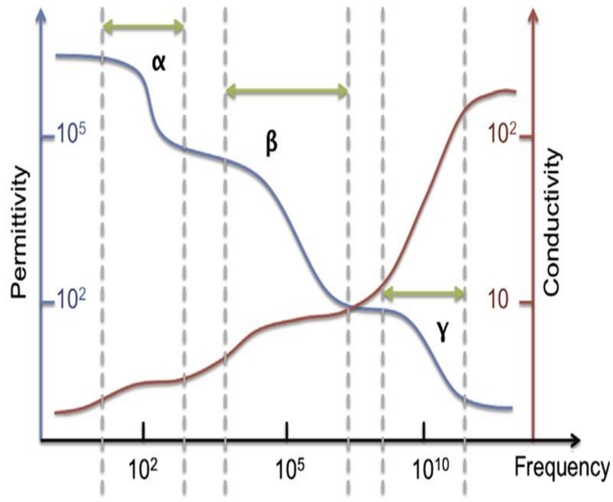

Figure 5. The dispersion curve of impedance in biological materials

For the bioimpedance characteristics of bacteria, alpha, beta, and gamma dispersion can be used, although there are some differences in the context of microorganisms. In general, the frequencies used are low, medium, and high frequencies [14]. The division between low, medium and high frequencies can vary depending on the context of its use. In this bacterial measurement, the frequency range is from 1 Hz to 100.000 Hz. Low frequencies range from 1 Hz to 1000 Hz, and high frequencies from 1000 Hz to 100.000 Hz.

Based on figures 4 and 5, the frequency range that is most dominant and shows significant changes in impedance values is the mid-frequency range of 100 Hz to 1000 Hz. Therefore, this graph can be categorized under beta dispersion. Beta dispersion provides insights related to the current still flowing around the cells but beginning to affect the space within the cell membrane. The frequency chosen in this measurement is 100 Hz, which shows the most significant changes, specifically at 100 Hz.

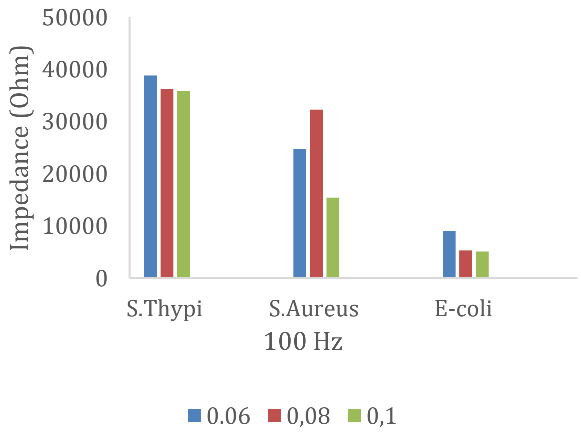

Figure 6. Graph 100 Hz

From the data provided and the accompanying graph, it is evident that the impedance of the three types of bacteria (*Salmonella typhi*, *Staphylococcus aureus* and *Escherichia coli)* varies depending on the sample mass used at a frequency of 100 Hz. Based on Figure 6, At 100 Hz, *Salmonella Typhi* has the highest impedance value, with a mass of 0,06 grams having an impedance of approximately 39.000 Ohms, 0,08 grams with an impedance of 35.000 Ohms, and 0,10 grams with an impedance of 34.000 Ohms. *Salmonella typhi* exhibits the highest impedance value among the bacteria, indicating greater resistance to electrical current at smaller masses [19]. Staphylococcus Aureus follows this with a mass of 0,06 grams having an impedance of 23.000 Ohms, 0,08 grams having a high impedance of 31.000 Ohms and 0,10 grams with an impedance of 15.000 Ohms. *Escherichia Coli*, with a mass of 0,06 grams, has an impedance of 9.000 Ohms, 0,08 grams has an impedance of 5.000 Ohms, and 0,10 grams has an impedance of 5.000 Ohms. The observed impedance variations in Staphylococcus aureus and Escherichia coli illustrate how cell mass and distribution impact resistance to electrical current at specific frequencies. A decrease in impedance with increased mass [20], may be attributed to alterations in cell interactions that facilitate the flow of electricity.

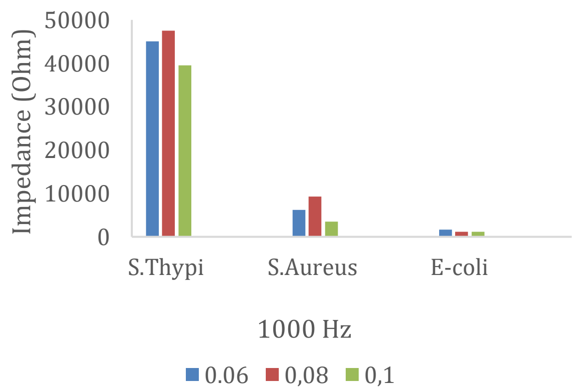

Figure 7. Graph 1000 Hz

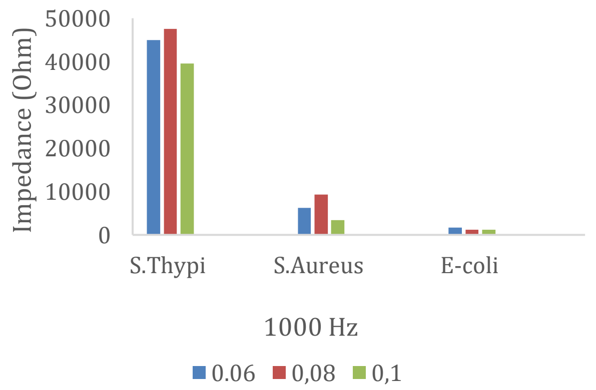

Figure 8. Graph 1000 Hz

Based on Figure 7, at a frequency of 1000 Hz, *Salmonella Typhi* has the highest impedance value. With a mass of 0,06 grams, the impedance is approximately 45.000 Ohms; with a mass of 0,08 grams, the impedance is 48.000 Ohms; and with a mass of 0,10 grams, the impedance is 40.000 Ohms. This is followed by *Staphylococcus Aureus*, with a mass of 0,06 grams having an impedance of 5.000 Ohms, 0,08 grams having a high impedance of 10.000 Ohms and 0,10 grams having an impedance of 3.000 Ohm. *Escherichia Coli*, with a mass of 0,06 grams, has an impedance of 2.000 Ohms, 0,08 grams has an impedance of 1.000 Ohms, and 0,10 grams has an impedance of 1.000 Ohms. The most effective frequency for differentiation is 100 Hz with a current of 10 uA. At 100 Hz, *Salmonella Typhi* has the highest impedance, followed by *Staphylococcus Aureus* and *Escherichia Coli*. In this impedance measurement, the same frequency was used for each bacterium [17]. The highest impedance value at 0.08 grams might be due to optimal bacterial density or growth conditions affecting electrical resistance. Additionally, it could be due to the specific interaction between the sample weight and the frequency of the applied electrical current. So, frequency affects the impedance value [19].

The differences among these three bacteria are as follows: *Salmonella Typhi* and *Escherichia Coli* are Gram-negative bacteria with a thin peptidoglycan layer but an additional outer membrane containing *lipopolysaccharides* [21], [22]. Although *Escherichia Coli* and *Salmonella Typhi* are Gram-negative, *Escherichia coli* has smooth *lipopolysaccharides* with long and diverse O-chains, providing better protection against lysis and antibiotics. In contrast, *Salmonella Typhi* has rough *lipopolysaccharides* without long O-chains, making it more susceptible to the complement system and antibiotics[9]. On the other hand, Staphylococcus Aureus is a Gram-positive bacterium characterized by a thick *peptigoglycan* layer but lacking an outer membrane [21], [23].

Conclusion

Electrical Impedance Spectroscopy can identify and compare *Escherichia coli, Staphylococcus Aureus, and Salmonella Typhi. Significant changes are observed at 100 Hz, where Salmonella Typhi shows the highest impedance (39.000 Ohms at 0,06 grams), while Escherichia coli* has the lowest (5.000 Ohms at 0.08 and 0,10 grams). Thus, Impedance is more influenced by the intrinsic characteristics of bacterial cells than by bacterial mass.

References

[1] Kurniati, I., & Kalsum, U. (2018). Analisis Pertumbuhan Escherichia Coli Dan Salmonella Typhi Pada Media Macconcey Agar (Mca) Dengan Penambahan Ekstrak Daun Jati (Tectona Grandis Lf). *Jurnal Analisis Biologi*, *2*(01).

[2] Febriza, M. A., & Adrian, Q. J. (2021). Penerapan Ar Dalam Media Pembelajaran Klasifikasi Bakteri. *Jurnal Bioeduin*, *11*(1), 10-18.

[3] Holderman, M. V., De Queljoe, E., & Rondonuwu, S. B. (2017). Identifikasi Bakteri Pada Pegangan Eskalator Di Salah Satu Pusat Perbelanjaan Di Kota Manado. *Jurnal Ilmiah Sains*, *17*(1), 13-18.

[4] Cita, Y. P. (2011). Bakteri Salmonella Typhi Dan Demam Tifoid. *Jurnal Kesehatan Masyarakat Andalas*, *6*(1), 42-46.

[5] Levani, Y., & Prastya, A. D. (2020). Demam Tifoid: Manifestasi Klinis, Pilihan Terapi Dan Pandangan Dalam Islam. *Al-Iqra Medical Journal: Jurnal*

Berkala Ilmiah Kedokteran, *3*(1), 10-16.

[6] Ruiz-Pérez, N. J., Sánchez-Navarrete, J., & Toscano-Garibay, J. D. (2021). Natural Products For Salmonellosis: Last Decade Research. In *Salmonella Spp.-A Global Challenge*. Intechopen.

[7] Purnamasari, N., & Fuadi, N. (2022). Uji Kandungan Bakteri Total Coliform Dan Escherichia Coli Air Tanah Di Kabupaten Pangkep. *Sainfis: Jurnal Sains Fisika*, *2*(1), 1-7.

[8] R. Falamy, E. Warganegara, Dan E. Apriliana. (2012). "Detection Of Coliform Bacteria At Cincau Hitam Market Snacks In Traditional And Supermarkets In Bandar Lampung City," *Major. J. Lampung Univ.*, Vol. Issn 2337-, Hal. 1–9,.

[9] M. Caroff Dan A. Novikov. (2020) "Lipopolysaccharides: Structure, Function And Bacterial Identification 0–9.

[10] N. Khairunnisa, K. Lisa Yuniati, A. St Fahirah Arsal, Dan R. Faisal Syamsu. (2023). "Efektifitas Ekstrak Daun Kemangi & Ekstrak Daun Sirih Merah Sebagai Anti Mikroba Staphylococcus Aureus Penyebab Furunkle," *Fakumi Med. J. J. Mhs. Kedokt.*, Vol. 3, No. 2, Hal. 106–111.

[11] Febyayuningrum, I. C., Rosyidah, R. A., & Aini, R. (2021). Kontaminasi Bakteri Alat Stetoskop Dengan Media Bap Dan Mca Di Ruang Penyadapan Darah Udd Pmi Kabupaten Sleman Diy. *Jurnal Ilmu Kedokteran Dan Kesehatan Indonesia*, *1*(3), 106-116.

[12] Sugianto, W. (2024). "Identifikasi Tingkat Kematangan Buah Pisang Berdasarkan Karakteristik Impedansi Listrik Dengan Metode Spektroskopi Impedansi Listrik Menggunakan Elektroda 4 Jarum," *Berk. Fis. Indones. J. Ilm. Fis. Pembelajaran Dan Apl.*, Vol. 15, No. 1, Hal. 24.

[13] Zhang, F., Jin, T., Hu, Q., & He, P. (2018). Distinguishing Skin Cancer Cells And Normal Cells Using Electrical Impedance Spectroscopy. *Journal Of Electroanalytical Chemistry*, *823*, 531-536.

[14] Abasi, S., Aggas, J. R., Garayar-Leyva, G. G., Walther, B. K., & Guiseppi-Elie, A. (2022). Bioelectrical Impedance Spectroscopy For Monitoring Mammalian Cells And Tissues Under Different Frequency Domains: A Review. *Acs Measurement Science Au*, *2*(6), 495-516.

[15] Nabila, F., Widodo, C. S., & Santoso, D. R. (2023). Correlation Of Whole Blood Cell Morphology On Electrical Impedance Spectroscopic Characteristics On The Severity Level Of Ischemic Stroke Patients. *International Journal Of Biological, Physical And Chemical Studies*, *5*(2), 40-49.

[16] Apipah, E. R. (2014). Sintesis Dan Karakteristik Membran Nilon Yang Berasal Dari Limbah Benang. *Jurnal Biofisika*, *10*(1).

[17] Putri, R. R. A., Sulistya, C., & Santoso, D. R. (2017). Analisis Nilai Impedansi Listrik Pada Daging Ikan Nila Yang Disimpan Dalam Lemari Es. *Indonesian Journal Of Applied Physics*, *6*(02), 117-124.

[18] Maulandari, E. Z. (2018). Analisis Hasil Pengukuran Impedansi Listrik Dengan Menggunakan Metode Injeksi Arus Tipe Floating Dan Howland. *Malang: Skripsi Ub*.

[19] Bobu, F. R. (2023). Analisis Karakteristik Nilai Impedansi Listrik Pada Daging Ikan Nila (Oreochhromis Nilaticus). *Optika: Jurnal Pendidikan Fisika*, *7*(1), 80-86.

[20] Sugianto, W. (2023). Kajian Awal Karakteristik Impedansi Listrik Larutan Nacl Pada Berbagai Konsentrasi Menggunakan Metode Spektroskopi Impedansi Listrik. *Magnetic: Research Journal Of Physics And It's Application Volume 3, No. 1 Maret 2023 E-Issn: 2775-8583*.

[21] Tirono, M., Abtokhi, A., & Samsu, F. (2018). Optimasi Medan Listrik Berpulsa Untuk Menurunkan Jumlah Bakteri Staphylococcus Aereus, Salmonella Sp., Dan Escherichia Coli Pada Susu.

[22] Silhavy, T. J., Kahne, D., & Walker, S. (2010). The Bacterial Cell Envelope. *Cold Spring Harbor Perspectives In Biology*, *2*(5), A000414.

[23] Nikolic, P., & Mudgil, P. (2023). The Cell Wall, Cell Membrane And Virulence Factors Of Staphylococcus Aureus And Their Role In Antibiotic Resistance. *Microorganisms*, *11*(2), 259.