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Bacterial Viability Analysis Using Multimodal Integration of Bioimpedance Spectroscopy and Spectrophotometry

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Abstract – *Monitoring bacterial viability is essential in food safety, healthcare, and environmental applications, particularly for evaluating microbial contamination and treatment effectiveness. This study aims to analyze the relationship between bioimpedance data and absorbance in determining the viability of Escherichia coli after an alcohol-induced lysis process. In addition, this study explores the potential integration of bioimpedance spectroscopy and spectrophotometry without requiring complex electrode modifications. Bioimpedance measurements were performed using the BioSMET system based on Electrical Impedance Spectroscopy (EIS) over a frequency range of 1 Hz–100 kHz. The measurement used Interdigitated Electrodes (IDE) without biosensor coating, following the principle that impedance spectroscopy can be applied for bacterial characterization and monitoring. Meanwhile, absorbance measurements were conducted at a wavelength of 260 nm, which is commonly associated with optical detection of biological materials. The E. coli samples were treated with alcohol concentrations of 0%, 10%, 35%, and 70%, followed by centrifugation to collect the supernatant for further measurement. Data analysis was carried out using the Multiple Linear Regression (MLR) method, with Log TPC values used as the reference for viable bacterial counts. The results show that a single impedance parameter had a limited correlation with bacterial viability, with an R^2 value of 0.2902. In contrast, the absorbance parameter showed a stronger correlation, with an R^2 value of 0.9447. Furthermore, the integration of bioimpedance and absorbance parameters using the MLR method produced the best performance, achieving an R^2 value of 0.9721 and an RMSE of 0.41. These findings indicate that the combination of bioimpedance spectroscopy and spectrophotometry provides a more representative assessment of bacterial viability than the use of a single measurement parameter.*

Keywords – *Bacterial viability; Escherichia coli; bioimpedance spectroscopy; spectrophotometry; multiple linear regression.*

I. INTRODUCTION

MONITORING bacterial populations is critical across various fields, including food safety, medical diagnosis, and environmental monitoring [1–3]. In the food industry, microbial contamination is a major cause of product spoilage and foodborne illness, leading to significant public health risks and economic losses. In clinical settings, the rapid and accurate assessment of bacterial viability is essential for guiding diagnosis, antibiotic selection, and infection control. In environmental applications, bacterial monitoring serves as an indicator of water quality, sanitation effectiveness, and ecosystem health. Consequently, the development of fast, reliable, and accessible methods for evaluating bacterial viability has become a continuous research priority.

Among the bacterial species commonly investigated, *Escherichia coli* is widely used as a model organism due to its well-characterized physiology, rapid growth rate, and dual role as both a commensal inhabitant of the human gut and an opportunistic pathogen. Its presence in food and water samples is frequently used as an indicator of fecal contamination and inadequate sanitary conditions [4]. Therefore, evaluating the viability of *E. coli* under various treatment conditions provides practical insights into the effectiveness of antimicrobial interventions and disinfection procedures.

One phenomenon frequently used to evaluate bacterial damage in microbiological studies is cell lysis, a condition in which the cell membrane ruptures and causes intracellular components to be released into the surrounding environment [3]. Through this process, cell death can be observed, particularly as a result of specific treatments such as alcohol exposure [5].

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Ethanol induces lysis by disrupting the lipid bilayer of the bacterial cell membrane and denaturing membrane-associated proteins, which leads to the leakage of cytoplasmic contents into the medium. The extent of cell damage and the corresponding release of intracellular components depend strongly on alcohol concentration, providing a controlled means to observe gradual changes in bacterial viability under well-defined experimental conditions.

Traditionally, bacterial quantification has relied on the Total Plate Count (TPC) method, which serves as the established gold standard [2]. Although TPC offers reliable enumeration of viable cells, it is labor-intensive and time-consuming, typically requiring 24–48 hours of incubation before colony counting can be performed. These limitations restrict its applicability in scenarios that demand rapid decision-making, such as in-line food processing, water quality monitoring, or point-of-care diagnostics. In response, several studies have employed regression-based approaches using a single measurement type, such as bioimpedance or spectrophotometry alone. However, each of these methods has inherent limitations when applied in isolation.

One emerging method is Electrical Impedance Spectroscopy (EIS), which is used to observe the electrical response of a sample across a broad frequency range. EIS operates by injecting a small alternating current into a sample and measuring the resulting complex impedance at different frequencies. At low frequencies, the current predominantly flows through the extracellular medium because the cell membrane acts as an insulating barrier; at higher frequencies, the current can penetrate the membrane and probe intracellular properties, a phenomenon commonly referred to as β-dispersion [6]. This frequency-dependent behavior makes EIS particularly suitable for monitoring bacterial conditions, since it can capture information about both membrane integrity and ionic redistribution in the surrounding medium.

In this study, measurements were conducted using the BioSMET, or Bioimpedance Spectroscopy Measurement System. The use of impedance spectroscopy allows non-destructive monitoring of the biophysical properties of a sample, enabling the observation of cellular changes during treatment [7, 8]. In general, this method can detect changes occurring both inside the cell and in the extracellular fluid [9, 10]. However, without surface modification or biosensor coating on the electrodes, the resulting signals reflect the overall condition of the medium. Consequently, it becomes difficult to distinguish whether the changes originate from live cells, dead cells, or lysis products [11]. Furthermore, at low frequencies, impedance signals are often influenced by electrode polarization effects, which can mask the biological response of the sample [12]. Therefore, selecting an appropriate frequency range is important to ensure that the measurements remain representative of the cellular state. Within specific ranges, impedance changes can be correlated with cell membrane conditions and ionic shifts in the medium, allowing lysis to be observed through an appropriate analytical approach.

In contrast, spectrophotometry measures the absorbance of a solution at specific wavelengths. In the context of cell lysis, a wavelength of approximately 260 nm is frequently employed because it corresponds to the absorption maximum of nucleic acids (DNA and RNA) released from damaged cells [13]. The OD_{260} measurement therefore provides a direct optical indicator of intracellular content leakage and has been widely used as a complementary technique for assessing membrane permeability. Nevertheless, this method is more representative of the post-lysis fluid and does not directly describe the overall condition of the cells, particularly during the early stages of damage when nucleic acid release remains limited.

Consequently, each method has its own strengths and limitations. EIS can capture changes related to both the cells and their surrounding environment, but it has difficulty differentiating the sources of the measured signals. Meanwhile, spectrophotometry is more sensitive to lysis products but does not describe the integral state of the cells. Therefore, combining these two methods has the potential to provide more comprehensive information. A multimodal approach that integrates electrical and optical parameters can offer a more holistic view of cellular conditions compared with the use of single parameters [14]. The integration of biophysical and optical data represents a multimodal strategy that can enhance the evaluation of bacterial viability [15].

Previous studies have generally utilized bioimpedance or spectrophotometry separately to estimate bacterial viability [10, 16]. While several investigations have demonstrated the feasibility of impedance-based bacterial detection using functionalized electrodes coated with antibodies, aptamers, or polymer films, such approaches often require complex fabrication processes and specialized reagents that increase cost and reduce accessibility. Similarly, advanced optical techniques such as Raman spectroscopy or fluorescence-based assays provide high sensitivity but rely on expensive instrumentation that is not always available in routine laboratory settings. To date, relatively few studies have systematically explored the integration of EIS and OD_{260} data using

bare Interdigitated Electrodes (IDE) for bacterial viability estimation, which represents the research gap addressed by this study.

This study implements a simple integration of bioimpedance data and OD_{260} absorbance using Interdigitated Electrodes (IDE) without biosensor modification. This approach maintains a simple measurement system while providing a more comprehensive estimation compared with the use of a single parameter. In this research, data from EIS and spectrophotometry were utilized simultaneously to observe the relationship between measured signal changes and the condition of *Escherichia coli* cells treated with alcohol. The primary focus is to analyze the correlation between impedance and absorbance data with viable cell counts, particularly under lysing conditions. The analysis was performed using Multiple Linear Regression (MLR) as a baseline approach to examine the interconnectivity between the measured parameters.

The novelty of this study lies in three main aspects. First, it demonstrates that a low-cost, non-functionalized IDE system can be effectively combined with conventional spectrophotometry to estimate bacterial viability across a wide range of lysis conditions, without requiring complex electrode modifications. Second, it provides a quantitative comparison between single-parameter and multimodal regression models, evaluated using R^2 and RMSE metrics, thereby offering a clear baseline for future multimodal viability studies. Third, it identifies an optimal working frequency through a sensitivity analysis performed across the entire EIS frequency range, providing practical guidance for the selection of analysis frequencies in subsequent bioimpedance-based bacterial monitoring applications.

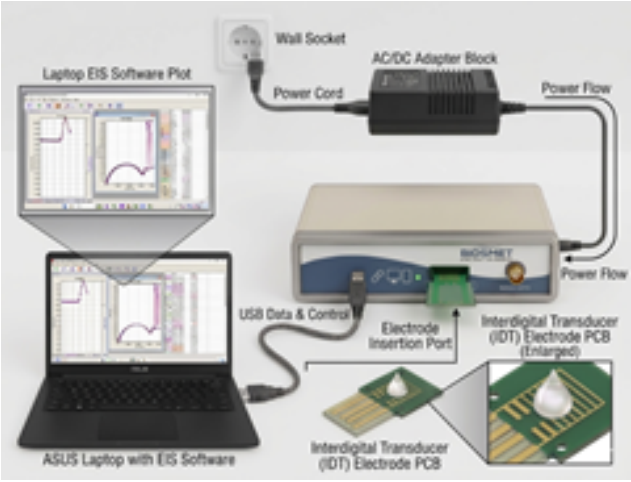

Figure 1: BioSMET measurement system configuration and sample drop method.

Through this approach, the combination of both methods is expected to provide a more complete description of the cellular state than when each method is used individually, without requiring complex electrode modifications. Further developments may involve electrode surface modification, for example by using polymer or lipid coatings, to enhance the sensitivity and specificity of detection toward specific biological conditions [17].

The remainder of this paper is organized as follows. Section II describes the research methods, including sample preparation, the measurement setup, and the analysis procedures. Section III presents the experimental results and discussion, including the frequency sensitivity analysis, single-parameter regression models, and the integrated multimodal model. Finally, Section IV summarizes the main findings and outlines potential directions for future research.

II. Research Methods

The primary instrument employed in this study was an Electrical Impedance Spectroscopy (EIS)-based measurement system using the BioSMET, or Bioimpedance Spectroscopy Measurement System, device [18]. This system was used to measure the impedance response of the samples over a frequency range of 1 Hz to 100 kHz. The frequency range was selected to observe the electrical response of the samples, including both the condition of the extracellular fluid and the changes in the cell membrane during the lysis process.

Measurements were performed using Interdigitated Electrodes (IDE) without any additional coating, hereafter referred to as bare electrodes [12]. The use of uncoated electrodes was intended to maintain a simple and straightforward measurement system. However, as a consequence, the resulting signals did not originate from a single component only. Instead, they represented a composite response from live cells, dead cells, and ions within the medium. Therefore, to obtain a more comprehensive understanding of the sources of signal changes, additional data from spectrophotometry were integrated into the analysis.

The measurement current was set to 10 mA. This value was selected to ensure stable signal readings with reduced noise while remaining within a safe operating range to prevent sample alteration, such as overheating or electrochemical reactions, during the measurement process [9]. The configuration of the EIS measurement system using BioSMET and the sample deposition method on the electrodes are shown in Fig. 1.

The samples used in this study consisted of pure cultures of *Escherichia coli* grown in Luria–Bertani (LB) medium. Prior to treatment, the cell concentration was standardized by measuring the optical density at a

wavelength of 600 nm, denoted as OD_{600}. This wavelength is commonly used to assess bacterial cell density based on light scattering, while minimizing interference from intracellular components such as proteins and nucleic acids. The OD_{600} value was adjusted to 0.280 to represent the active growth state, or exponential phase, thereby ensuring that the initial condition of the samples remained relatively uniform.

Cell lysis was induced using 96% alcohol with final concentration variations of 0%, 10%, 35%, and 70% (v/v). These variations were selected to represent cellular conditions at different stages, ranging from untreated control samples and initial membrane disruption to more dominant cell damage [3]. This range allowed the observation of incremental changes in cellular conditions. The detailed volume composition for each treatment variation is presented in Table 1.

Table 1: Variation of lysis agent dose and sample volume composition.

Target Concentration	Bacterial Volume (mL)	Alcohol 96% Volume (mL)	Aquadest Volume (mL)
0% (Control)	5.0	0.0	5.0
10%	5.0	1.1	3.9
35%	5.0	3.7	1.3
70%	2.7	7.3	0.0

Each mixture was homogenized using a vortex mixer until thoroughly blended, followed by incubation for 10 minutes at room temperature. This incubation time was selected to allow sufficient interaction between alcohol and the bacterial cell membrane prior to measurement. Following incubation, the samples were centrifuged at 10,000 rpm for 5 minutes to separate cellular debris from the liquid phase. The supernatant was then collected as the test sample because it contained intracellular components released during the lysis process, such as ions and nucleic acids. This supernatant was subsequently used for both bioimpedance and spectrophotometric measurements.

In the spectrophotometric measurements, a specific blank solution was prepared for each concentration variant according to the solvent composition of the respective sample. This procedure was applied to ensure that the absorbance readings were not influenced by solvents, particularly alcohol, so that the measured values more accurately represented the components released as a result of lysis.

To maintain data consistency, each treatment was performed in four replicates, resulting in a total of 16 observation data points. The number of viable cells was determined using the Total Plate Count (TPC) method through serial dilution until colony counts within a countable range were obtained. These TPC values served as the reference for analyzing the relationship between impedance and absorbance data. The overall stages of the research are summarized in Fig. 2.

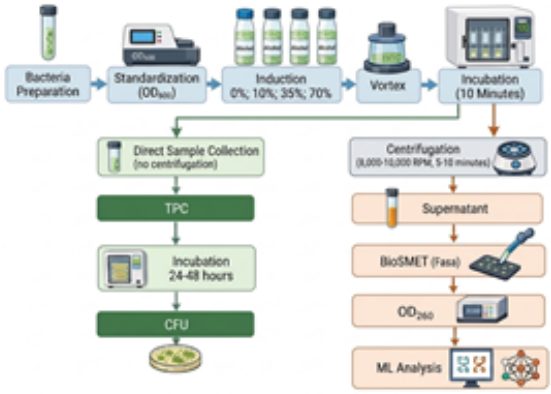

Figure 2: Flowchart of the research stages.

Data analysis was conducted to evaluate the relationship among bioimpedance data, OD_{260} absorbance, and the number of viable cells expressed as Log TPC. In this study, Multiple Linear Regression (MLR) was employed as an initial approach to integrate two different types of measurement data [19]. The regression method was selected because it allows the trend between parameters to be observed in a straightforward and interpretable manner. Furthermore, previous studies have indicated that computational processing of impedance data can significantly improve the estimation quality of cellular conditions [20].

The analysis phase began with a correlation test across the entire measured frequency range from 1 Hz to 100 kHz. This test was conducted to determine the specific impedance frequency that showed the clearest relationship with changes in viable cell counts while maintaining better signal stability against electrode polarization effects. The impedance value at the selected frequency was then used as one of the variables in the regression model, as expressed in Eq. (1).

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 \tag{1}$$

In Eq. (1), Y represents the estimated Log TPC value, X_1 denotes the bioimpedance value, and X_2 denotes the absorbance value at OD_{260}. The coefficients β_1 and β_2 were determined by minimizing the Root Mean Square Error (RMSE) in order to obtain optimal predictive performance.

The use of a linear model at this stage was based on previous studies indicating that changes in cell counts can be approximately related to changes in the electrical properties of the medium under certain conditions [9]. However, under specific circumstances, particularly at high concentrations, the relationship may deviate from linearity. Therefore, the model in Eq. (1) was used as a preliminary approach to observe the correlation trends among parameters rather than as an absolute prediction model.

This analysis focused on the interconnection between impedance and absorbance data in describing cellular conditions, particularly during the lysis process. To assess the quality of the model, the coefficient of determination, R^2, and the Root Mean Square Error (RMSE) were used as evaluation parameters to indicate the extent to which the estimated results aligned with the measured TPC data.

III. Results and Discussion

The results of this study indicate that the application of alcohol at different concentrations induced changes in the biophysical response of *Escherichia coli*. Alcohol acts as a lysing agent that can disrupt the lipid and protein structures of the cell membrane, thereby increasing membrane permeability [1]. This condition leads to the leakage of intracellular components, such as ions and nucleic acids, into the surrounding medium. The release of these components subsequently alters the electrical characteristics of the sample as measured by Electrical Impedance Spectroscopy (EIS) and influences the absorbance values in OD_{260} measurements. The relationship between impedance and frequency at different alcohol concentrations is shown in Fig. 3.

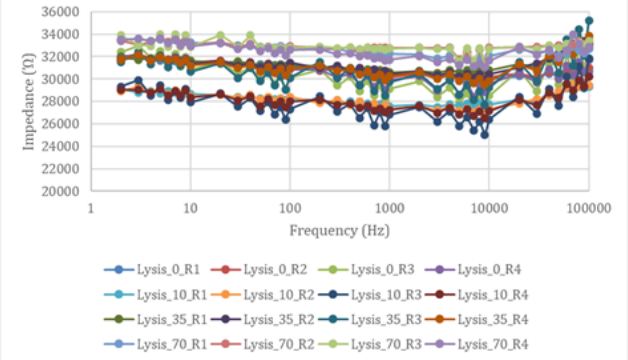

Figure 3: Graph of the relationship between impedance and frequency at various alcohol concentrations.

Based on Fig. 3, each alcohol concentration produced a distinct impedance pattern across the entire measured frequency range. In general, changes in impedance values were influenced by shifts in the composition of the medium after the lysis and centrifugation processes. Since the samples measured in this study were supernatants obtained after centrifugation, the impedance response primarily represented changes in the electrical properties of the medium caused by ions, proteins, and intracellular components released from bacterial cells.

At low frequencies, the impedance response remained influenced by electrode polarization and the distribution of ions near the surface of the Interdigitated Electrodes (IDE). Nevertheless, the low-frequency range remains important for analysis because changes in medium conductivity due to ion release from lysis are more dominantly detected in this region. Meanwhile, at higher frequencies, the influence of electrode polarization decreases, resulting in a more stable measurement response toward changes in sample conditions [9].

The data in Fig. 3 were obtained from four alcohol treatment variations, with four biological replicates for each treatment. The curve patterns across replicates showed relatively similar trends, indicating that the observed impedance changes were more strongly influenced by alcohol treatment than by measurement variation.

At the 10% alcohol concentration, impedance values tended to decrease compared with the control group. This condition indicates the beginning of cell membrane disruption, which caused intracellular ions to leak into the medium and increased solution conductivity. At the 35% concentration, the impedance change was more pronounced than that of the previous treatment, indicating a more intensive lysis process and a higher release of intracellular components into the supernatant.

However, at the 70% alcohol treatment, impedance values tended to increase again across several frequency ranges. In addition to ion distribution, this condition may also be related to protein denaturation and aggregation caused by high alcohol concentration [10]. Changes in the composition of biomolecules in the medium can affect the dielectric constant of the solution, causing the impedance response to no longer change linearly with the number of viable bacteria [21]. This indicates that the relationship between impedance and the level of cell damage is not always linear. Under severe lysis conditions, the presence of membrane fragments, denatured proteins, and changes in ion distribution within the medium can alter the dielectric properties of the solution, resulting in a more complex impedance response [22].

These measurement results demonstrate that the EIS system using IDE without a biosensor layer is still capable of detecting sample changes caused by the lysis process. However, because the electrodes used are non-selective, the obtained impedance response represents a composite of various components in the medium, including ions, cell debris, and other dissolved biomolecules. Nevertheless, impedance spectroscopy offers the advantage of label-free detection, allowing direct monitoring of bacterial conditions without additional electrode modification [7]. Therefore, OD_{260} spectrophotometry measurements were used as a supporting parameter to describe the release of intracellular

components during lysis.

Subsequently, to determine the most representative working frequency for changes in bacterial viability, a sensitivity analysis was performed across all measured frequency points. This analysis aimed to evaluate the capability of each frequency to distinguish changes in impedance response among lysis treatments. In addition to considering the correlation with Log TPC values, frequency selection also accounted for the sensitivity of impedance changes and the stability of the measurement response. The results of this sensitivity analysis are shown in Fig. 4.

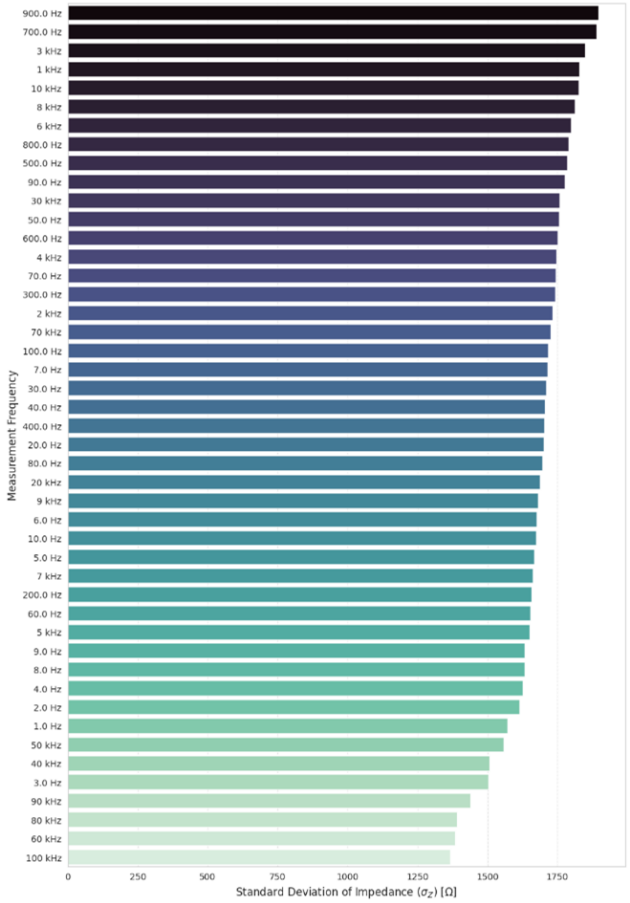

Figure 4: Sensitivity analysis of bioimpedance features to the number of viable bacteria.

The analysis presented in Fig. 4 was conducted to evaluate the sensitivity of impedance response across 48 measured frequency points with respect to changes in bacterial conditions due to alcohol treatment. Frequency sensitivity was evaluated based on the variation in impedance response between treatments, represented by the standard deviation of impedance, σ_Z. A higher σ_Z value indicates a greater capability of a specific frequency to distinguish changes in sample condition resulting from the bacterial lysis process.

Based on the analysis results, several low-to-mid frequencies exhibited relatively high sensitivity, such as 900 Hz, 700 Hz, 3 kHz, and 10 kHz. Among all measured frequencies, 900 Hz showed the highest σ_Z value and was therefore selected as the primary working frequency for subsequent analysis.

The high sensitivity at low frequencies suggests that changes in medium composition due to the lysis process are more dominantly detected within this range. At low frequencies, the impedance response is more significantly influenced by ion distribution and changes in the conductivity of the medium surrounding the electrodes [12]. Since the measured samples were supernatants obtained after centrifugation, the release of ions and dissolved biomolecules due to membrane damage contributed more substantially to impedance changes compared with the influence of intact cell structures.

Although low frequencies remain affected by electrode polarization effects, the high variation in response between treatments indicates that these frequencies still have a robust ability to differentiate changes in sample conditions caused by lysis. Consequently, 900 Hz was chosen as the working frequency for analyzing the relationship between impedance and bacterial viability in the next stage.

Once the working frequency was determined, the relationship among bacterial viability, impedance at 900 Hz, and OD_{260} absorbance values was analyzed, as summarized in Table 2.

Table 2: Measurement results of Log TPC, bioimpedance at 900 Hz, and absorbance at OD_{260} under various alcohol concentrations.

Sample Code	Log TPC (CFU/mL)	Impedance at 900 Hz	OD_{260}
Lysis_0_R1	9.730	30540.110	0.088
Lysis_0_R2	9.710	30520.330	0.086
Lysis_0_R3	9.750	28112.880	0.089
Lysis_0_R4	9.720	29057.770	0.087
Lysis_10_R1	7.270	27520.770	0.186
Lysis_10_R2	7.250	27695.770	0.182
Lysis_10_R3	7.300	25812.440	0.189
Lysis_10_R4	7.280	27009.660	0.185
Lysis_35_R1	2.890	30545.880	0.250
Lysis_35_R2	2.920	30725.440	0.246
Lysis_35_R3	2.850	28512.110	0.253
Lysis_35_R4	2.900	29894.730	0.249
Lysis_70_R1	2.820	32380.440	0.262
Lysis_70_R2	2.800	32818.540	0.259
Lysis_70_R3	2.840	32631.280	0.265
Lysis_70_R4	2.810	31627.920	0.261

Based on Table 2, the increase in alcohol concentration led to a decrease in Log TPC values, indicating a reduction in the number of viable bacteria due to the cell lysis process. In the control group, Log TPC values ranged from 9.71 to 9.75 Log CFU/mL, which then decreased to approximately 7.25–7.30 Log CFU/mL at the 10% alcohol treatment. A more significant reduction was observed at concentrations of 35% and 70%, where Log TPC values fell within the range of

2.80–2.92 Log CFU/mL. These results demonstrate that higher alcohol concentrations negatively affect bacterial viability due to cell membrane damage and disruption of the physiological state of the bacteria.

The impedance values at 900 Hz exhibited a different trend compared with the Log TPC values. In the 10% alcohol treatment, impedance tended to decrease relative to the control. This indicates that the release of ions and intracellular components resulting from membrane disruption made the medium more conductive, thereby reducing electrical resistance. However, at higher alcohol concentrations, particularly 35% and 70%, impedance values increased again in some samples. These findings suggest that the relationship between impedance and the number of viable bacteria is not entirely linear under severe lysis conditions.

In contrast to impedance, the absorbance values at OD_{260} showed a consistent upward trend as alcohol concentration increased. Absorbance values rose from approximately 0.086–0.089 in the control group to 0.259–0.265 in the 70% alcohol treatment. This increase indicates a higher accumulation of nucleic acids and other intracellular components released into the medium due to cell membrane damage. Consequently, the OD_{260} parameter is more representative of the presence of lysis products than of the overall electrical condition of the medium.

The results in Table 2 indicate that impedance and absorbance parameters have distinct response characteristics toward the bacterial lysis process. Impedance is more sensitive to changes in the electrical properties of the medium caused by membrane disruption and ion distribution, whereas OD_{260} better describes the release of genetic material from cell lysis. Therefore, both parameters were utilized simultaneously to obtain a more comprehensive representation of bacterial viability compared with the use of a single parameter.

As a preliminary stage in analyzing inter-parameter relationships, the correlation between impedance at 900 Hz and bacterial viability is illustrated in Fig. 5, while the single-impedance-based regression results are presented in Table 3.

Although the 900 Hz frequency exhibited the highest sensitivity to impedance changes based on the analysis in Fig. 4, the linear relationship between a single impedance parameter and bacterial viability at this frequency still demonstrated limited performance. Therefore, 900 Hz was used to evaluate the capability of a single impedance parameter in representing changes in bacterial viability during the lysis process. The resulting linear regression model is expressed in Eq. (2).

$$y = -0.000773x + 28.6363 \quad (2)$$

As shown in Eq. (2), the coefficient of determination was $R^2 = 0.2902$, with an RMSE value of 2.4888. The relatively low R^2 value indicates that the relationship between impedance at 900 Hz and Log TPC was not fully linear. This result shows that a single impedance parameter still has limitations in describing the biological changes occurring during the bacterial lysis process.

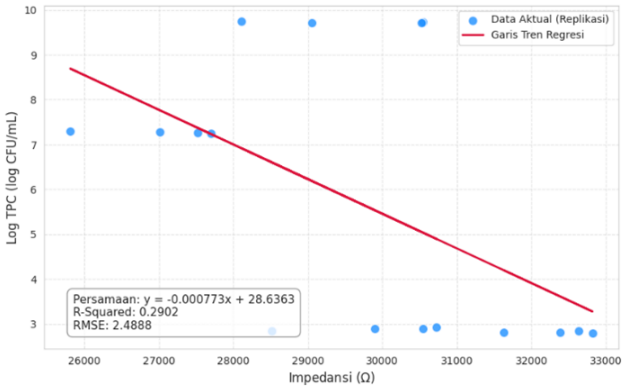

Figure 5: Relationship between Log TPC and impedance value at 900 Hz.

Table 3: Comparison of actual and predicted Log TPC values based on impedance parameters.

Sample Code	Impedance Value	Log TPC	TPC Prediction	Difference
Lysis_0_R1	30540.110	9.730	9.621	0.109
Lysis_0_R2	30520.330	9.710	9.703	0.007
Lysis_0_R3	28112.880	9.750	10.211	-0.461
Lysis_0_R4	29057.770	9.720	10.043	-0.323
Lysis_10_R1	27520.770	7.270	6.629	0.641
Lysis_10_R2	27695.770	7.250	6.737	0.513
Lysis_10_R3	25812.440	7.300	6.956	0.344
Lysis_10_R4	27009.660	7.280	6.800	0.480
Lysis_35_R1	30545.880	2.890	3.388	-0.498
Lysis_35_R2	30725.440	2.920	3.495	-0.575
Lysis_35_R3	28512.110	2.850	3.791	-0.941
Lysis_35_R4	29894.730	2.900	3.595	-0.695
Lysis_70_R1	32380.440	2.820	2.451	0.369
Lysis_70_R2	32818.540	2.800	2.453	0.347
Lysis_70_R3	32631.280	2.840	2.271	0.569
Lysis_70_R4	31627.920	2.810	2.684	0.126

The limitations of this linear relationship are evident in the prediction results shown in Table 3. The single-impedance-based regression model showed differences between actual and predicted values across several treatment conditions. In the control and low alcohol concentrations, the prediction difference remained relatively small. However, under higher lysis conditions, particularly in the 35% and 70% alcohol treatments, the error values tended to increase.

The increase in error indicates that the impedance response was not only influenced by the number of viable bacteria but also by changes in medium composition during the lysis process. This is consistent with the results in Fig. 5, where several data points under high lysis conditions deviated from the regression line due to the influence of cell debris and changes in the

dielectric properties of the medium on the electrical response of the system.

These results show that the use of a single impedance parameter still has limitations in comprehensively describing the biological condition of the sample. Therefore, in the next stage, the integration of impedance and absorbance parameters was performed using the Multiple Linear Regression (MLR) approach to evaluate the relationship of both parameters with Log TPC simultaneously. The relationship between OD_{260} absorbance and bacterial viability is shown in Fig. 6.

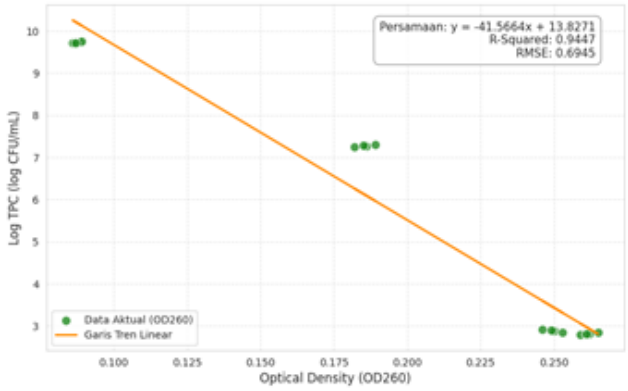

Figure 6: Relationship between Log TPC and absorbance value at OD_{260}.

Based on Fig. 6, an increase in the OD_{260} absorbance value was followed by a decrease in bacterial Log TPC value. This indicates that as more intracellular components were released into the medium, the number of viable bacteria tended to decrease. The resulting linear regression model is shown in Eq. (3).

$$y = -41.5664x + 13.8271 \quad (3)$$

As presented in Eq. (3), the coefficient of determination was $R^2 = 0.9447$, with an RMSE of 0.6945. This value indicates that approximately 94.47% of the variation in bacterial viability can be explained by changes in the OD_{260} absorbance value. The high R^2 value shows that the absorbance parameter had a better linear relationship with Log TPC compared with the single impedance parameter.

Based on the data distribution in Fig. 6, the measurement points appeared closer to the regression line than those in the single impedance model. This condition indicates that changes in absorbance had a more consistent relationship with the decrease in the number of viable bacteria. Biologically, the increase in absorbance at a wavelength of 260 nm is related to the release of nucleic acids, including DNA and RNA, and other intracellular components into the medium due to alcohol-induced cell membrane damage [4]. The greater the level of cell damage, the more intracellular material is released into the medium, thereby increasing the OD_{260} value.

Table 4: Comparison of actual and predicted Log TPC values based on absorbance parameters.

Sample Code	Absorbance Value	Log TPC	TPC Prediction	Difference
Lysis_0_R1	0.088	9.730	9.802	-0.072
Lysis_0_R2	0.086	9.710	9.883	-0.173
Lysis_0_R3	0.089	9.750	9.761	-0.011
Lysis_0_R4	0.087	9.720	9.843	-0.123
Lysis_10_R1	0.186	7.270	5.815	1.455
Lysis_10_R2	0.182	7.250	5.978	1.272
Lysis_10_R3	0.189	7.300	5.693	1.607
Lysis_10_R4	0.185	7.280	5.856	1.424
Lysis_35_R1	0.250	2.890	3.211	-0.321
Lysis_35_R2	0.246	2.920	3.374	-0.454
Lysis_35_R3	0.253	2.850	3.089	-0.239
Lysis_35_R4	0.249	2.900	3.252	-0.352
Lysis_70_R1	0.262	2.820	2.723	0.097
Lysis_70_R2	0.259	2.800	2.845	-0.045
Lysis_70_R3	0.265	2.840	2.601	0.239
Lysis_70_R4	0.261	2.810	2.764	0.046

Although the single absorbance model demonstrated a better linear relationship, the results in Table 4 indicate that this model still had limitations under certain treatment conditions. At the 10% alcohol concentration, the prediction error appeared higher than in other treatments, with discrepancies exceeding 1 Log CFU/mL in several replicates. This condition suggests that changes in absorbance values were not yet fully capable of representing the decline in bacterial viability during the initial phase of cell damage.

In the early phase of lysis, some bacteria may have undergone physiological disruption and reduced viability, while their cell membranes had not yet experienced comprehensive damage. Consequently, the release of genetic material into the medium remained limited, and the change in OD_{260} absorbance did not increase proportionally to the reduction in viable bacterial counts. This condition resulted in relatively high prediction errors at the 10% alcohol treatment.

Conversely, at higher alcohol concentrations of 35% and 70%, the error values tended to be smaller. These results indicate that the OD_{260} parameter is more sensitive in detecting advanced lysis conditions, where intracellular components have been extensively released into the medium. Under these conditions, the change in absorbance becomes more representative of the level of cell damage occurring.

When compared with the impedance method, both parameters exhibited distinct but complementary response characteristics. Bioimpedance was more sensitive to initial changes in medium condition due to shifts in ion distribution and cell membrane disruption, whereas OD_{260} provided a more stable relationship during advanced lysis when the release of intracellular components increased significantly. Therefore, the integration of bioimpedance and OD_{260} absorbance pa-

rameters through the MLR approach was performed to obtain a more representative bacterial viability estimation model compared with the use of single parameters. The model validation graph is shown in Fig. 7.

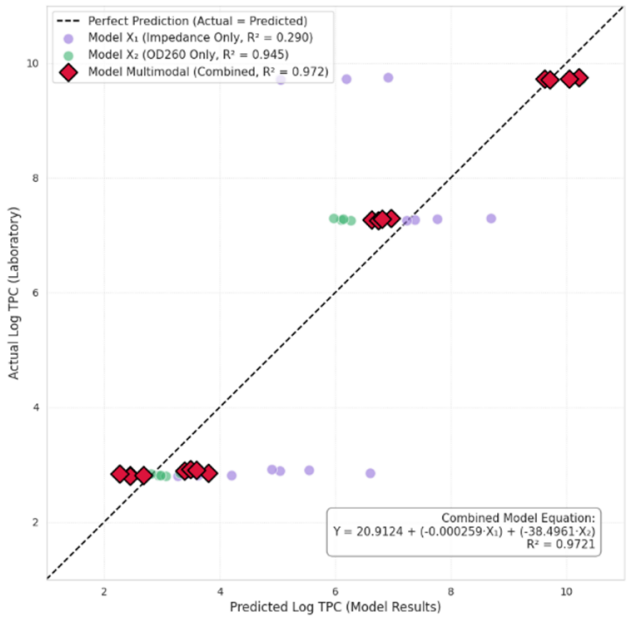

Figure 7: Model performance validation graph showing actual versus predicted values.

Based on Fig. 7, the multimodal model demonstrated superior predictive performance compared with the single-parameter models, as indicated by the distribution of data points lying closer to the perfect prediction line. The resulting multiple linear regression model is expressed in Eq. (4).

$$Y = 20.9124 + (-0.000259X_1) + (-38.4961X_2) \quad (4)$$

In Eq. (4), X_1 represents the impedance value, while X_2 represents the OD_{260} absorbance value. The negative coefficients for both variables indicate that an increase in either impedance or absorbance was followed by a decrease in bacterial viability. The difference in coefficient values was influenced by the different measurement scales between impedance and absorbance; therefore, it does not directly reflect the magnitude of each parameter's individual contribution.

Quantitatively, the single impedance model showed lower performance with $R^2 = 0.2902$, characterized by a data distribution that remained relatively distant from the reference line, particularly under high lysis conditions. The single absorbance model showed a better relationship with $R^2 = 0.9447$, yet it still exhibited deviations during the lysis transition phase, specifically at the 10% alcohol concentration. The integration of both parameters through the MLR approach resulted in improved model performance with $R^2 = 0.9721$, accompanied by a more consistent distribution of data points along the reference line. These results demonstrate that the multimodal approach was capable of mitigating the limitations of each individual parameter, thereby producing a more stable estimation of bacterial viability.

The comparison of actual and predicted Log TPC values based on the integration of impedance and absorbance data is presented in Table 5.

Table 5: Comparison of actual and predicted Log TPC values based on impedance and absorbance data integration.

Sample Code	Impedance Value	Absorbance Value	Log TPC	TPC Prediction	Difference
Lysis_0_R1	30540.110	0.088	9.730	9.623	0.107
Lysis_0_R2	30520.330	0.086	9.710	9.706	0.004
Lysis_0_R3	28112.880	0.089	9.750	10.213	-0.463
Lysis_0_R4	29057.770	0.087	9.720	10.045	-0.325
Lysis_10_R1	27520.770	0.186	7.270	6.632	0.638
Lysis_10_R2	27695.770	0.182	7.250	6.741	0.509
Lysis_10_R3	25812.440	0.189	7.300	6.958	0.342
Lysis_10_R4	27009.660	0.185	7.280	6.803	0.477
Lysis_35_R1	30545.880	0.250	2.890	3.386	-0.496
Lysis_35_R2	30725.440	0.246	2.920	3.493	-0.573
Lysis_35_R3	28512.110	0.253	2.850	3.796	-0.946
Lysis_35_R4	29894.730	0.249	2.900	3.593	-0.693
Lysis_70_R1	32380.440	0.262	2.820	2.449	0.371
Lysis_70_R2	32818.540	0.259	2.800	2.451	0.349
Lysis_70_R3	32631.280	0.265	2.840	2.269	0.571
Lysis_70_R4	31627.920	0.261	2.810	2.682	0.128

The analysis in Table 5 shows that the integration of impedance and absorbance parameters helped reduce the prediction deviations that appeared in the single-parameter models, particularly during the lysis transition phase. In the single absorbance model, the error value at the 10% alcohol concentration reached 1.607 Log CFU/mL. This condition indicates that changes in absorbance did not fully represent the decline in bacterial viability during the initial phase of cell damage.

Under these conditions, the bioimpedance parameter provided additional information through changes in the electrical properties of the medium and cell membrane before significant release of genetic material occurred. Conversely, under advanced lysis conditions, particularly at 35% and 70% alcohol concentrations, the OD_{260} parameter exhibited a more stable response due to the higher release of intracellular components into the medium. Thus, both parameters demonstrated complementary response characteristics in describing changes in bacterial conditions during the lysis process.

Biophysically, impedance represents changes in the electrical properties of the medium caused by membrane disruption and ion distribution, whereas OD_{260} represents the release of nucleic acids due to cell lysis. The integration of these two parameters resulted in a more comprehensive representation of bacterial viability conditions compared with the use of a single parameter [9].

The reduction in error values in the MLR model demonstrates that the integration of bioimpedance and absorbance was capable of mitigating the limitations of

each individual parameter. The impedance parameter was more sensitive to early changes in medium condition and ion distribution, while OD_{260} more accurately represented the release of intracellular material under advanced lysis conditions. The combination of these two parameters produced an estimation model that was more stable across various bacterial lysis conditions.

This study was conducted using a limited sample size. Therefore, further testing with a larger dataset and broader sample variation is required to assess the consistency and generalizability of the resulting model.

IV. Conclusion

This study demonstrates that the alcohol-induced lysis process causes measurable changes in both bioimpedance response and OD_{260} absorbance values in *Escherichia coli*. The single impedance parameter showed a limited relationship with bacterial viability, with an R^2 value of 0.2902. Meanwhile, the OD_{260} absorbance parameter exhibited a stronger correlation, with an R^2 value of 0.9447. The integration of both parameters using the Multiple Linear Regression (MLR) method yielded the best estimation performance, with an R^2 value of 0.9721 and an RMSE of 0.41.

These results indicate that the combination of bioimpedance and absorbance provides a more comprehensive representation of bacterial viability than the use of single parameters. Moreover, this approach can be implemented without requiring complex electrode modifications, thereby offering a simple, practical, and effective strategy for bacterial viability estimation.

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