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DEVELOPMENT OF A MACHINE LEARNING MODEL FOR PREDICTING *ESCHERICHIA COLI* GROWTH UNDER DIFFERENT ENVIRONMENTAL CONDITIONS

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ABSTRACT
Escherichia coli (*E. coli*), a Gram-negative bacterium predominantly inhabiting the intestines of warm-blooded animals, including humans, encompasses both benign and pathogenic strains. The ability of this strain to persist and proliferate in food matrices underscores the critical importance of effective control measures and predictive tools in ensuring food safety across the food production and distribution chain. This research investigated the development of an Artificial Neural Network (ANN) model for predicting Colony Forming Units (CFU) of *E. coli* based on selected environmental factors such as temperature and pH. The study involved the collection of CFU data under varying conditions, with temperatures ranging from 25 °C to 50 °C and pH levels from 2 to 12. The ANN model demonstrated a high predictive accuracy, achieving an R-squared value of 98%, indicating strong correlations between predicted and actual CFU values. The results showed that the optimal growth temperature for *E. coli* was 35 °C and pH of 7 (neutral), where the predicted CFU closely matched the actual count. Additionally, the model proved effective across a range of conditions, confirming its reliability as a tool for predicting microbial growth. These findings underscore the potential application of the ANN model in fields such as food safety, microbiology, and environmental monitoring, providing a valuable resource for controlling bacterial populations in various settings.

Keywords: Machine learning model, *Escherichia coli,* Growth, Environmental conditions.

1.0 INTRODUCTION

Escherichia coli (*E. coli*), a Gram-negative bacterium predominantly inhabiting the intestines of warm-blooded animals, including humans, encompasses both benign and pathogenic strains. Certain pathogenic variants, such as *E. coli* 0157 pose significant public health risks worldwide due to their potential to cause severe food borne illnesses. These strains' ability to persist and proliferate in food matrices underscores the critical importance of effective control measures and predictive tools in ensuring food safety across the food production and distribution chain. (Smith *et al.,* 2020, Mordecai *et al*., 2024).

Predicting microbial growth dynamics, especially of pathogens like *E. coli,* is paramount for safeguarding food products. Environmental factors such as temperature, pH, moisture content, nutrient availability, and the presence of competing microorganisms profoundly influence *E. coli* growth dynamics within food products (Jones and Brown, 2019). Understanding these environmental influences is fundamental for implementing preventive strategies and interventions aimed at reducing the incidence of foodborne illness outbreaks (Musa *et al*., 2023).

Traditional methods for assessing microbial growth typically involve labor-intensive culturing in controlled laboratory settings, which may not fully capture the complex interactions between environmental variables influencing microbial behavior in real-world conditions. In contrast, machine learning (ML) techniques offer a promising avenue for enhancing predictive modeling of microbial growth. ML algorithms excel in analyzing vast datasets containing diverse environmental parameters and microbial responses, thereby uncovering intricate non-linear relationships and patterns that traditional statistical methods might overlook (FAO, 2018).

Recent research underscores the effectiveness of ML models in predicting microbial growth across various food matrices. These models integrate multiple environmental variables simultaneously, providing more accurate and timely predictions of microbial behavior compared to conventional approaches (Smith *et al.*, 2020). By harnessing ML capabilities, researchers can develop predictive models that not only bolster food safety management but also optimize production processes and ensure regulatory compliance within the food industry.

In recent years, machine learning has been introduced as a comprehensive approach, enabling complex data analysis and future predictions in a short amount of time. Furthermore, a distinction can be made between three types of machine learning applications, namely supervised, semi-supervised and unsupervised learning. The first category, supervised learning, is based on predicting which class the input belongs to after the model has been trained on a labeled data set. A method that often is considered logical and straightforward. On the other hand, when unsupervised learning is used, the aim is often not to obtain a distinct label on the input but to identify different patterns that the data contains (Mahesh, 2020). Lastly, semi-structures learning corresponds to a combination of the two where a small part of the data is labelled and a larger part is un-labelled (Van Engelen and Hoos, 2020).

Microbiology focuses on studying the activity of microorganisms, exploring the characteristics, culture conditions, and detection methods of microflora, taking its essence (discovering, utilizing, improving, and protecting beneficial microorganisms), and

removing its dross (preventing, controlling, or transforming harmful microorganisms). Thus, it is available for science and benefits mankind (Hanage, 2014; Ha and Devkota, 2020).

Recently, the main research hotspots in microbiology include community classification and its environmental role (Zhang *et al*., 2021), regulation of gut microbiome and host interactions (Turnbaugh *et al*., 2007; Jones *et al*., 2014; Malla *et al*., 2018; Ruff *et al*., 2020), development of pathogenic microorganisms and drug vaccines (Shahbaaz *et al*., 2016; Moos *et al*., 2017; Zhu *et al*., 2020), and trying to dilute the boundaries between microbiome and genome editing, molecular modification, ecology and resource utilization, biocatalysis, and synthesis (Stres and Kronegger, 2019; Galloway-Pena and Hanson, 2020). In addition, microbiology and multiomics (including genomics, epigenomics, transcriptomics, proteomics, and metabolomics) have combined and developed a variety of multiscale emerging fields (Liang *et al*., 2021).

The understanding of microorganisms started from microbial cell morphology and physiological and biochemical characteristics to microbial genotype identification at the nucleic acid and protein levels, and chemical analysis methods based on cell chemical composition analysis and numerical classification methods relying on the level of computational biology have also been established successively. The rapid progress in the discipline of microbiology is inseparable from the update of observation methods or techniques in the same period (Galloway-Pena and Hanson, 2020). With the advent of the Big Data era, the pressing questions for researchers have gradually evolved into how to quickly and efficiently filter/condense this exponential growth of information to obtain generalized quality data and how to transform the massive data of microbiota into easily understood and visualized knowledge. Compared to traditional research with insufficient data or purely experimental techniques that cause trouble, such as cognitive bias, low reproducibility, and long-time span, the modern microbiology research process is more likely to incorporate new technologies and big data methods to do this better and right.

Besides supervised, semi-supervised, and unsupervised machine learning, a common distinguishment within supervised machine learning is classification models and regression models. The section below discusses the aims and differences between the two groups, Osisanwo *et al.* (2017) describe machine learning classification as a method for making a model take data-driven decisions to divide the data into different distinct groups based on linear combinations of their feature values. In other words, this means using input vectors including features for hyperplane decisions to classify the input. Generally, classification is favorable for tasks where the data points have many variable properties including similarities and differences but still a fundamental quality that identifies them (El Naqa and Murphy, 2015). Hence, classification interprets those properties and classifies the new data point with the proper label. Examples, where classification is used, are Jajodia and Garg (2019) who made a model that classifies whether images contain dogs or cats, and Li *et al.* (2020) who identified heart diseases using machine learning classification in e-healthcare. Moreover, linear classifiers often are beneficial where fast decisions are required. Osisanwo *et al.* (2017) also highlight that quite a few models are adequate for classification which among others include Logistic Regression, Support Vector Machine, Random Forest, and Neural Networks.

In contrast to classification, regression as machine learning algorithms that estimate a specific value to a task such as future energy load using other information including sunlight and wind. Furthermore, Maulud and Abdulazeez (2020) mean that regression can be used for two specific cases. Firstly, for forecasting and prediction where future values are predicted based on dependent variables. Secondly, regression is used to determine correlations between independent and dependent variables such as air temperature and water temperature which clearly are dependent (Aransiola *et al*., 2024). Consequently, regression is adequate for finding correlations between a specific variable and a data set containing points with other features. As a result, machine learning regression has been used in successful studies previously. Examples, which aim for different research fields are Zhang and Hong (2021), who proposed an approach for electric forecasting with support vector regression, Pereira and Cerqueira (2022) who used machine learning regression methods to forecast hotel demand for revenue management, and lastly predicted stock prices using sliding-window metaheuristic-optimized machine learning regression.

2.0 MATERIALS AND METHODS

The study utilized a variety of materials and apparatus, including soil samples, nutrient agar, EMB agar, distilled water, and general laboratory supplies such as aluminium foil, cotton wool, sterile containers, petri dishes, and protective gear. Key equipment includes the autoclave, gas cooker, incubator, and weigh balance.

2.1 Collection and preparation of sample

For the collection and preparation of samples, 5g of soil was collected from the surface at Federal University of Technology Minna (FUT) in front of Microbiology laboratory, which was stored in a sterile container. The experiment was preceded with a serial dilution process, beginning with the preparation of distilled water as a diluent. Test tubes were labelled from 1 to 8 to indicate dilution factors ranging from $10^{-1}$to 10^{-8}. The 0.5g of soil sample was mixed with distilled water to create a stock solution. A sequential dilution was performed by transferring 1 mL of the stock solution into the first test tube containing 9 mL of distilled water, then repeating the procedure for the subsequent tubes to achieve the desired dilutions.

2.1.1 Media preparation

The media preparation involved the use of 20g of nutrient agar and EMB agar, which was prepared strictly in accordance with the manufacturer's instructions and sterilized using an autoclave (Merk Manual, 2005).

2.1.2 Culturing

During the culturing phase, nutrient agar was poured into petri dishes and left to solidify. Following this, 0.5ml of the diluted sample was spread onto the agar surface and incubated at 37°C for 18-24 hours (American Public Health Association, 2005). The organisms that grow were isolated by sub-culturing them onto fresh nutrient agar plates using a streaking technique to obtain pure isolates.

2.1.3 Biochemical tests and morphology

Biochemical tests and morphological observations were conducted to further characterize the isolates. Gram staining was used to determine the reaction of the bacteria, while a series of IMViC tests (Indole, Methyl Red, Voges-Proskauer, and Citrate utilization)

helped to identify and differentiate bacterial species. Pure isolates were inoculated onto EMB agar and incubated under various conditions, including different temperatures and pH levels, for 24-48 hours to study their growth patterns as per APHA (2005).

Environmental conditions such as temperature and pH was carefully controlled and varied using incubators and buffers.

2.1.5 Growth measurement
The colony counting was employed as described in Merck Manual (2005) to estimate the number of viable cells. Additionally, microscopic techniques and digital counters was used for precise cell counting.

2.2 Development of the Machine Learning Model

2.2.1 Data collection
Data was collected from various publicly available online databases, including Kaggle, and Google Dataset Search. The datasets encompassed measurements of *E. coli* growth under diverse environmental conditions, including temperature, pH, salinity, and nutrient concentration. The selected datasets were downloaded in CSV format and control both quantitative and qualitative data. (Jones & Brown, 2019).

2.2.2 Data Preprocessing
The raw data was pre-processed to ensure consistency and reliability. Missing values were identified and handled using appropriate methods such as imputation with the mean or median values, or deletion of records with excessive missing data. Outliers were detected using statistical techniques such as the z-score method and were either corrected or removed based on their impact on the dataset.

Feature selection was conducted to identify relevant variables for predicting *E. coli* growth. Environmental factors such as temperature, pH, and nutrient concentration retained, while irrelevant features were discarded. The data were normalized using min-max scaling to bring all features into a common range, thereby facilitating the training process. The dataset was divided into training, validation, and test sets in a ratio of 70:15:15, ensuring that each set was representative of the overall data distribution. (Jones & Brown,2019).

2.3 Feature Engineering
Feature engineering was performed to enhance the predictive power of the model. Interaction terms between environmental factors was created to capture potential synergistic effects. Additionally, dimensionality reduction techniques, such as Principal Component Analysis (PCA), was employed to reduce the feature space and eliminate multicollinearity. (FAO, 2018).

2.4 Model Development
Multiple machine learning algorithms was selected for model development, including Linear Regression, Decision Trees, Random Forest, Support Vector Machines, and Neural Networks. These models were chosen based on their suitability for regression tasks and their ability to handle complex relationships between variables.

The models were trained using the training dataset. Hyperparameter tuning was conducted using Grid Search and Random Search to identify the optimal settings for each algorithm. The performance of the models was evaluated using the validation set, with metrics such as Mean Squared Error (MSE) and R^2 score employed to assess accuracy and goodness-of-fit. (Mahesh,2020).

2.5 Model Optimization
Further optimization was achieved through k-fold cross-validation, which was utilized to mitigate overfitting and ensure the robustness of the models. This process involved dividing the training set into k subsets, training the model on k-1 subsets, and validating it on the remaining subset.
The optimized model was tested on the independent test (experimental data) set to evaluate its generalization capability. Performance metrics, including MSE and R^2 score, were calculated and compared to those obtained from the validation phase. Statistical analyses were conducted to determine the significance of the results and to validate the model's predictive accuracy (Ruder,2016).

RESULTS AND DISCUSSION

Table 1: Colony Morphology of the isolated organism

Mixed Culture	Sub Culture	Isolate
Small, smooth, circular, raised, flat colonies with a greyish, reddish pink colour	Smaller, circular, greyish-white colonies	Smooth, circular, whitish and clear colonies were observed

Table 2: Biochemical Tests for the identification of *Escherichia coli*

Gram Staining	**-**	**Rod-shaped**	**pink**	
Indole	**+**	**NA**	**pink**	
Methyl Red	**+**	**NA**	**red**	***Escherichia coli***
Voges-Proskauer	**-**	**NA**	**No change**	
Citrate	**-**	**NA**	**No change**	

Indole Test was Positive, indicated by the presence of a pink color. **Methyl red test** also indicated a positive result shown by the development of a red color. Whereas, **Voges-Proskauer test**, shows a negative result, as there was no color change. The **citrate test,** also shows a negative result, with no observable color change. The positive results for the Indole and Methyl Red tests further confirmed the presence of *E. coli*, as these are standard tests for this organism. The negative results for the Voges-Proskauer and Citrate tests also align with its expected biochemical profile.

2.6 Confirmation on EMB Agar
Culture on EMB agar yielded small circular colonies with a **greenish metallic sheen**, confirming the presence of ***E. coli***. The observation of a greenish metallic sheen on EMB agar solidifies the identification, as this is characteristic of *E. coli* colonies due to their lactose fermentation ability.

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Table 3: Count of *E. coli* at different Temperature

Parameter	Number of Colonies	Colony forming unit (Cfu/g)
Temperature	-	-
^{o} 4 C		
^{o} 25 C	30	6.0×10^{5}
^{o} 35 C	387	7.74×10^{5}
^{o} 50 C	100	1.99×10^{5}

Table 4: Count of *E. coli* at different pH

Parameter	Number of Colonies	Colony forming unit (Cfu/g)
pH	-	-
2		
7	400	7.99×10^{5}
12	-	-

The CFU data supports the findings regarding temperature and pH. At 35°C and neutral pH, the CFU count was (7.74×10^{5}) and (7.99×10^{5}) respectively which are the highest, reaffirming the optimal conditions for *E. coli* growth. Lower CFU values at 25°C, 50°C, and extreme pH values reflect suboptimal conditions where growth is either slowed or inhibited.

At **4°C**, no colony was observed, appearing as straight-line. At **25°C**, Moderate growth, with around **30 small colonies**, while at **35°C**, Optimal growth observed, with **387 colonies**, larger and more crowded. At **50°C**, Reduced growth, with **100 smaller colonies**. Growth patterns across the tested temperatures suggest that 35°C is the optimal growth temperature for *E. coli*, which corresponds to its natural habitat in the human gut where temperatures are approximately the same. At 4°C, growth was limited, confirming that *E. coli* does not thrive under cold conditions. Similarly, at 50°C, growth was reduced, indicating thermal stress at higher temperatures. This temperature-related behavior aligns with the expected physiological limits of mesophilic bacteria (Smith *et al*., 2020).

2.7 Effect of pH on Growth

At **pH 12**, no growth was observed, whereas at **pH 7 (neutral)**, significant growth with **400 colonies** was observed. At **pH 2**, Scanty growth was observed with colonies not countable, appearing as a straight line. The impact of pH on *E. coli* growth follows a predictable trend, with neutral pH (pH 7) supporting the highest colony growth. This finding highlights the organism's preference for neutral environments, which is consistent with its natural adaptation to the neutral pH of the human digestive system. At extreme pH values, such as pH 2 (acidic) and pH 12 (basic), growth was significantly hindered. This indicates that *E. coli* cannot survive in highly acidic or alkaline environments, likely due to disruption of its cellular processes

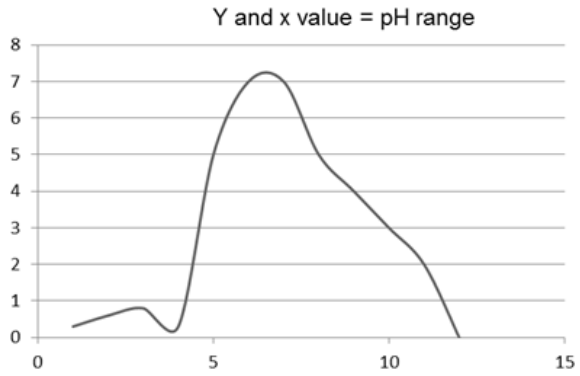

Figure 1: Graph illustrating the growth of *E. coli* under different pH range

The pH graph represents a typical bacterial growth curve, which signifies the lag phase (period of adaptability), log phase (exponential growth phase), the stationary phase (phase of rapid decline) and the death phase

Model Performance

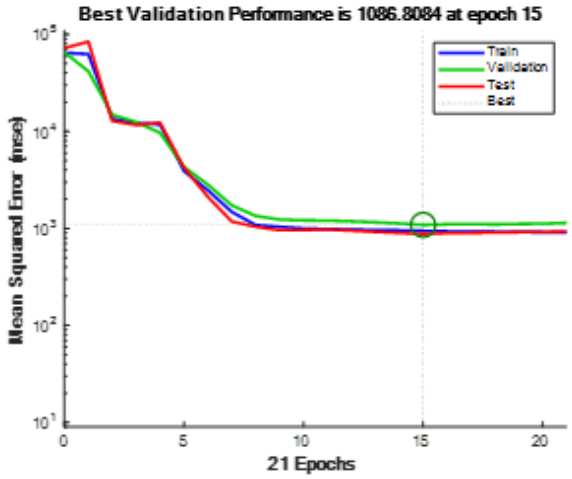

Figure 2: Mean Squared Error vs. Epochs for Training, Validating and Test Sets

This figure illustrates the Mean Squared Error (MSE) for the training, validation, and test sets over 21 epochs, highlighting the model's learning trajectory. Initially, MSE was high across all datasets, but it decreased significantly during the early epochs as the model started to grasp the underlying patterns. By the 15th epoch, the MSE for both validation and test sets stabilized and aligne closely with the training MSE, indicating that the model has reached optimal performance and demonstrates effective generalization. The absence of divergence among the curves suggests that the model avoids overfitting, and early stopping contributed to achieving the best performance by the 15th epoch. The plot confirms that the model fits the data well and generalizes effectively to new, unseen data, with no further improvements observed in subsequent epochs.

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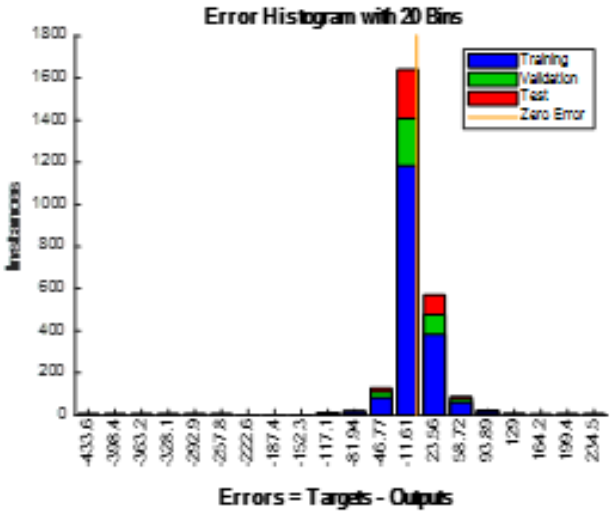

Figure 3: Error Distribution Histogram for Training Validation and Test Sets

This displays a histogram of prediction errors across the training, validation, and test sets of the neural network model. The histogram shows that most errors, particularly in the training set (blue), are concentrated around zero, indicating that the model's predictions are largely accurate for most data points. Although some instances exhibit larger errors, as shown by the bars at non-zero values, the overall distribution remains similar across the validation (green) and test sets (red). This similarity suggests that the model generalizes well and does not experience significant overfitting. The "Zero Error" line represents the ideal error value of zero, which the model approximates closely in most cases, demonstrating strong performance.

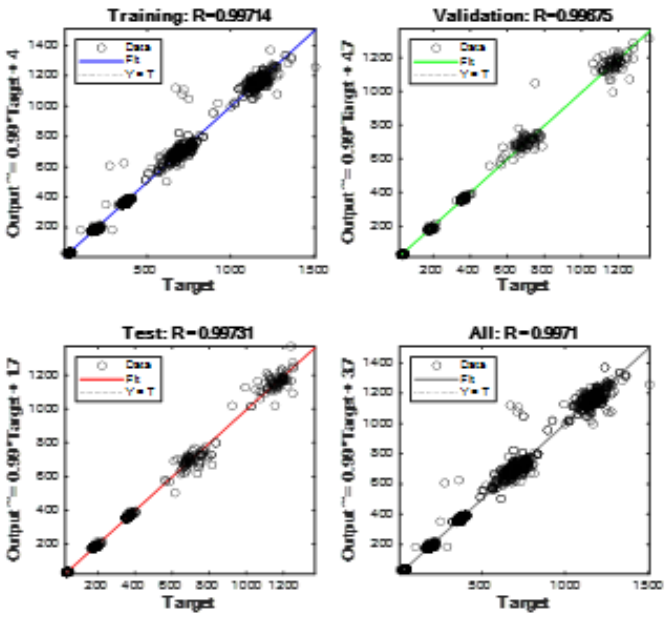

Figure 4: Regression Plot

A scatter plot of actual versus predicted values, visualizes how well the neural network model's predictions align with actual values. Ideally, points should cluster around the 45-degree diagonal line, which denotes perfect predictions. While many points are indeed close to this line, reflecting accurate predictions, some deviations are noticeable, indicating instances where the model's predictions diverged significantly from the actual values. These deviations highlight opportunities for further refinement of the model.

The model's overall Root Mean Square Error (RMSE) is 42.9509, reflecting the average deviation between predicted and actual values. Although a lower RMSE would typically indicate a better fit, an RMSE of 42.9509 might be significant depending on the scale of the response variable, particularly if the range of values is relatively small. On the other hand, the Overall R-squared value of 0.9880 demonstrates that the model explains 98.80% of the variance in the data. This high R-squared value indicates that the model captures most of the variability in the actual data, suggesting a very strong fit. Overall, these metrics suggest that the model performs well with high accuracy in predicting the response variables, though there is potential for minor improvements.

Table 5: Total *E. coli* count actual and predicted

Condition	Actual (Cfu/g)	Predicted (Cfu/g)
25°C	6.0×10^5	5.94×10^5
35°C	7.74×10^5	7.76×10^5
50°C	1.99×10^5	1.93×10^5
pH7	7.99×10^5	7.96×10^5

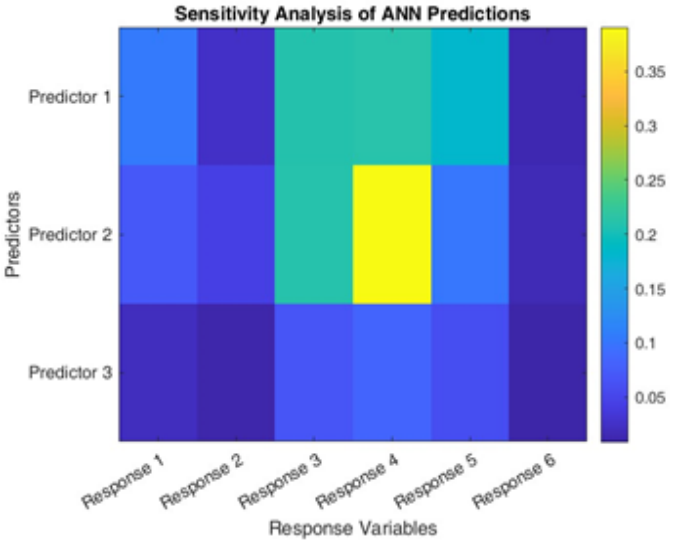

Figure 5: Sensitivity analysis of the ANN prediction

This illustrates the impact of different predictors on the model's responses. Predictor 2 exerts the most significant influence on Response 4, as shown by the bright yellow color, indicating that even small changes in Predictor 2 can lead to substantial variations in Response 4 predictions. Predictor 1 has a moderate effect on

Responses 2 and 3, while Predictor 2 also moderately influences Responses 3 and 5, as reflected by the greenish-blue shades. In contrast, Predictor 3 has minimal impact across all responses, represented by the dark blue color, suggesting that changes in this predictor have a negligible effect on the model's outputs. This analysis provides insights into which predictors are most and least influential, helping to guide future model refinement efforts.

Conclusion

This study successfully developed an Artificial Neural Network (ANN) model for predicting Colony Forming Units (CFU) of *E. coli* under varying environmental conditions, particularly temperature and pH. The model demonstrated high accuracy, with an R-squared value of 98%, indicating strong predictive capabilities. The predictions closely matched actual CFU values across a range of conditions, with minimal deviations observed. The optimal temperature for E. coli growth was confirmed at 35°C, where the model's predicted CFU of 7.76×10^5 closely aligned with the actual value of 7.74×10^5. At non-optimal conditions such as 50°C and extreme pH values, the model still performed well, showing its robustness across different environmental factors. This suggests that the ANN model is a reliable tool for predicting bacterial growth in controlled environments, making it useful in applications related to microbiology, food safety, and environmental monitoring..

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