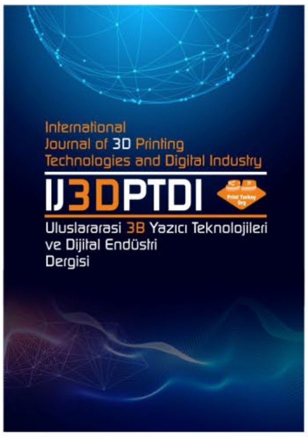

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PREDICTION PERFORMANCE OF DECISION TREE INDUCERS ON AUGMENTED BACILLUS CEREUS GROWTH DATA

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ABSTRACT

Accurate prediction of microbial growth is of great importance in critical areas such as food safety and environmental sciences. In this study, a hybrid of mathematical methods and machine learning-based approaches are used to model the growth dynamics of foodborne pathogen *Bacillus cereus*. Since the use of mathematical models alone does not sufficiently cover the non-linear data structure of bacterial systems, better results are obtained when hybrids are used together with machine learning methods. We examine the results of five different tree-based models for predicting the growth of Bacillus cereus, namely Fine Tree, Medium Tree, Coarse Tree, Ensemble Boosted Trees and Ensemble Bagged Trees. We evaluate each model with performance metrics such as Root Mean Square Error (RMSE), Mean Square Error (MSE), R^2 and Mean Absolute Error (MAE). The results show that the Ensemble Bagged Trees model performs the best, with a validation RMSE of 0.0094 and an R^2 value of 0.9995. Also, the Fine Tree model has an R^2 value of 0.9990. In general, ensemble methods offer significant advantages in prediction accuracy.

Keywords: Data Augmentation, Machine Learning, Microbial Growth Prediction, Tree-Based Models

1. INTRODUCTION

Accurate prediction of microbial growth is crucial across disciplines such as food safety, agriculture, and environmental sciences. Bacillus cereus, a significant foodborne pathogen, poses serious health risks due to its ability to produce enterotoxins that cause gastrointestinal illness [1-2]. Understanding its growth under various environmental conditions is essential for effective risk mitigation. Key aspects include:

○ Food safety relevance: Accurate modeling of B. cereus growth is critical to prevent foodborne outbreaks and ensure consumer protection [3-4].
○ Agricultural impact: Microbial dynamics influence crop health and soil quality, affecting agricultural productivity [5-6].
○ Environmental significance: Modeling microbial behavior contributes to understanding ecological interactions and managing microbial risks [7-8].

Recent studies emphasize the integration of modeling techniques to address the multifactorial and complex nature of microbial growth [9-10]. Historically, microbial growth has been described using mathematical models, which are typically based on simplified assumptions regarding environmental conditions [11]. However, these models present several limitations [12-13]:

○ Limited complexity: Classical models often fail to capture the nonlinear and dynamic interactions inherent in biological systems [14-15].
○ Low adaptability: Their static structures hinder generalization across diverse environmental conditions or food matrices [16].
○ Decreased accuracy: These models struggle to accurately reflect the effects of rapid or synergistic changes on microbial behavior [17].
○ Data limitations: Traditional models are generally trained on limited and homogeneous datasets, which impedes their

ability to represent extensive and variable real-world data [18].

- Complex influence of environmental factors: Factors such as temperature, pH, and humidity interact in variable and combined ways that classical models do not fully represent [1,19].
- Challenges in parameter estimation: Determining model parameters is often difficult, and inaccurate estimations adversely affect model performance [14].
- Insufficient model flexibility: The ability of the model to rapidly adapt to environmental or experimental changes is limited [15].
- Neglect of biological diversity: Variations in growth characteristics among different strains or species are inadequately accounted for in classical models [3,14].

These limitations impede accurate and reliable predictions of microbial growth, thereby complicating risk management and decision-support processes.

The aim of this study is to enhance the accuracy of predicting Bacillus cereus microbial growth dynamics and to improve the generalizability of modeling approaches. To achieve this, an integrative framework combining mathematical modeling with machine learning techniques has been developed to more effectively capture the complex, nonlinear, and dynamic interactions inherent in biological systems. In particular, decision tree-based and ensemble methods have been employed to increase model robustness and predictive reliability across diverse datasets and environmental conditions. Additionally, polynomial regression techniques have been utilized to model higher-order functional relationships, while data augmentation strategies have expanded the diversity and size of the training dataset. Through this integrative approach, the study seeks to provide more accurate and reliable predictions of Bacillus cereus growth under various real-world scenarios, thereby contributing to the development of effective decision-support tools in food safety, agricultural productivity, and environmental risk management.

2. MATERIAL and METHOD

2.1. Bacterial Strain and Culture Conditions

In this study, Bacillus cereus was employed as the model bacterial strain. The initial cultivation and preparation steps were conducted under controlled and sterile conditions to ensure experimental reliability:

- Bacillus cereus colonies were grown on petri dishes containing Nutrient Agar and incubated at 37 °C for 24 hours to allow colony formation. Two to three distinct colonies were then selected and transferred to sterile tubes containing Nutrient Broth.
- The selected colonies were vortexed vigorously to produce a homogeneous suspension, which was subsequently incubated overnight at 37 °C with shaking at 130 rpm to promote optimal growth and obtain sufficient cell density.
- After incubation, the optical density at 660 nm (OD_{660}) was measured using a UV spectrophotometer to assess the bacterial growth level and confirm suitability for further experimentation.
- Two milliliters of the overnight culture were inoculated into 50 mL of fresh Nutrient Broth in sterile 250 mL Erlenmeyer flasks and incubated at 37 °C with shaking at 130 rpm. All procedures were performed aseptically to prevent contamination.
- Inoculations were conducted in triplicate to ensure data reproducibility. The initial OD_{660} was recorded, followed by hourly measurements to monitor the growth kinetics of Bacillus cereus, as OD_{660} is a standard indicator for bacterial cell density while minimizing interference from the culture medium [17].

2.2. Data Augmentation with Polynomial Regression Model

In study the growth rate of Bacillus cereus is a fourth-order polynomial regression model was developed to characterize the growth dynamics of *Bacillus cereus* based on systematically collected experimental data:

Table 1. Summary of Bacillus cereus Growth Data, Augmentation Method, and Model Performance Metrics (Statistical metrics between measured data and augmented data)

Description	Details
Initial Dataset	9 data points including time, temperature, and bacterial count, collected under controlled laboratory conditions to construct the initial growth model.
Method	Data augmentation was performed using a Polynomial Regression Model to expand the dataset and enhance model robustness.
Expanded Dataset	Dataset increased to 401 data points to improve model precision and generalizability, capturing environmental influences on bacterial growth more comprehensively.
Model Performance Metrics	- Sum of Squared Errors (SSE): 0.0082 - Root Mean Square Error (RMSE): 0.0452 - Coefficient of Determination (R^2): 0.9951 - Adjusted R^2: 0.9901 - Degrees of Freedom for Error (DFE): 4

Metrics	time (observed)	time (augmented)	Bacillus cereus (observed)	Bacillus cereus (augmented)
min	0	0	0.054	0.038
max	8	8	1.187	1.201
mean	4	4	0.741	0.754
std dev	2.738	2.318	0.455	0.403
count	9	401	9	401

The dataset metrics indicate that the observed time values range from a minimum of 0 to a maximum of 8, with a mean of 4 and a standard deviation of 2.738, based on 9 data points. After augmentation, the time values retain the same minimum and maximum (0 and 8, respectively) and mean of 4, while the standard deviation slightly decreases to 2.318 across 401 data points. For Bacillus bacterial counts, the observed values span from 0.054 to 1.187 with a mean of 0.741 and a standard deviation of 0.455 based on 9 observations. The augmented Bacillus counts range from 0.038 to 1.201, with a mean of 0.754 and a standard deviation of 0.403 over 401 data points.

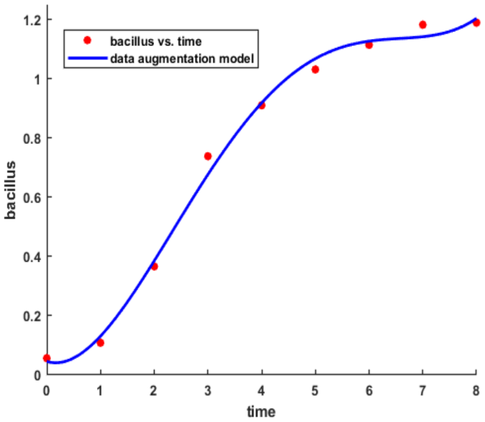

Figure 1. Measured data and augmented data

Figure 1 illustrates the original measured values of *Bacillus cereus* growth alongside the synthetically generated data obtained through data augmentation techniques. The augmented data extends the range and density of the dataset, enhancing the model's ability to generalize and improving prediction performance across varying conditions.

2.3. Tree-Based Machine Learning Models

The dataset consisted of 401 observations in total. Of these, 341 samples (~85%) were used for model training and validation via 5-fold cross-validation, while 60 samples (~15%) were reserved for independent testing. We use five different machine learning models to model the growth dynamics of Bacillus cereus: Fine Tree, Medium Tree, Coarse Tree, Ensemble Boosted Trees and Ensemble Bagged Trees. We choose each model for its distinct algorithmic advantages in capturing non-linear relationships and patterns in the dataset as follows in Figure 2:

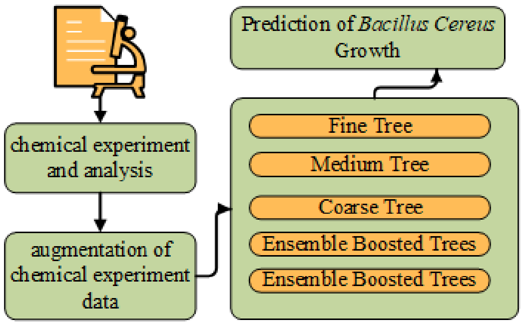

Figure 2. Prediction of Bacillus Cereus Growth with Machine Learning Based Models

Table 2 presents a comparative overview of five tree-based machine learning models used to predict the growth dynamics of Bacillus cereus, highlighting their structural complexity, key characteristics, overfitting risks, and optimal use cases. Each model offers distinct advantages in capturing non-linear patterns within the dataset, depending on its algorithmic design. While simpler models such as the Coarse Tree generalize well on linearly separable and low-dimensional data, more complex models like the Fine Tree and ensemble methods provide improved accuracy on intricate, non-linear datasets. Ensemble Boosted Trees and Ensemble Bagged Trees, in particular, demonstrate enhanced predictive performance by reducing bias and variance, respectively. Therefore, selecting the appropriate model requires careful consideration of both the data characteristics and the desired balance between model complexity and generalization capability.

The hyperparameters of the decision tree–based regression models employed in this study played a decisive role in determining model complexity, generalization capacity, and predictive accuracy. The Fine Tree model was configured with high complexity, using the Mean Squared Error (MSE) as the split criterion, a minimum parent node size (MinParent) of 10, a minimum leaf size (MinLeaf) of 4, and a maximum number of splits (MaxSplits) of 340. All predictor variables were considered for splitting (NVarToSample = all), and pruning was enabled with the MSE as the pruning criterion. The Medium Tree model was designed with MinParent set to 24 and MinLeaf set to 12, achieving a balanced trade-off between complexity and generalization. The Coarse Tree model, with MinParent set to 72 and MinLeaf set to 36, generated fewer branches, resulting in a simpler structure with higher generalization capability but reduced capacity to capture intricate relationships. Among the ensemble approaches, the Ensemble Bagged Trees model utilized 30 weak learners (NLearn = 30) with bootstrap aggregation (bagging) and a learning rate of 1.0, combining the independently trained trees through averaging. The Ensemble Boosted Trees model also used 30 weak learners but adopted a sequential improvement strategy via the LSBoost method with a learning rate of 0.1, whereby each subsequent tree was fitted to reduce the residual errors of its predecessor. These hyperparameter configurations contributed to the superior predictive performance of high-complexity tree structures and ensemble methods, particularly in terms of accuracy and generalization, compared with single decision tree models.

Table 2. Tree-Based Machine Learning Models for Bacillus cereus Growth

Model	Model Complexity	Key Characteristics	Overfitting Risk	Best Use Case
Fine Tree Model	High	Produces deep, complex trees; captures subtle interactions between time and environmental factors.	High	Nonlinear, Complex datasets
Medium Tree Model	Moderate	Balances complexity and performance; reduces risk of overfitting while maintaining sufficient predictive power.	Medium	Moderately complex datasets
Coarse Tree Model	Low	Simple and shallow structure; generalizes well; less prone to overfitting in simpler datasets.	Low	Small, Linearly separable datasets
Ensemble Boosted Trees	High (with sequential depth)	Sequentially corrects errors of prior models; reduces bias and improves prediction accuracy on complex datasets.	Medium	Complex, Nonlinear datasets
Ensemble Bagged Trees	Moderate to High	Uses bootstrap samples; trees trained independently; final prediction is average of individual predictions; reduces variance.	Low	Nonlinear, Noisy datasets

3. RESULTS AND DISCUSSIONS

The results of this study reveal notable differences in accuracy and error metrics between various tree-based models in predicting the target variable. The Ensemble Bagged Trees model, which has the highest overall accuracy among the tested models, calculate an RMSE of 0.009377553 in the validation set and an RMSE of 0.006517267 in the test set, as well as an R^2 value of 0.999465892 in the model validation set and 0.999725126 in the test set in Table 3.

Table 3. Tree regression models error and approximation values

Model	Type	RMSE	MSE	R^2	MAE	Pearson Correlation (r)
Fine Tree	Validation	0.01399	0.00019	0.99881	0.00947	0.9993
	Test	0.01239	0.00015	0.99900	0.00937	
Medium Tree	Validation	0.03084	0.00095	0.99422	0.02055	0.9975
	Test	0.02493	0.00062	0.99597	0.01728	
Coarse Tree	Validation	0.06947	0.00482	0.97068	0.05083	0.9856
	Test	0.05610	0.00314	0.97962	0.03899	
Ensemble Boosted Trees	Validation	0.03593	0.00129	0.99215	0.03102	0.9922
	Test	0.03459	0.00119	0.99225	0.03002	
Ensemble Bagged Trees	Validation	0.00937	0.00008	0.99946	0.00606	0.9997
	Test	0.00651	0.00004	0.99972	0.00453	

The RMSE values of the Fine Tree model are 0.013993197 in the validation set and 0.012392232 in the test set. The calculated R^2 values are 0.998810721 for the validation set and 0.999006194 for the test set. These values indicate that the model has a high capacity to capture variability in the data. These findings show that the Fine Tree model is effective in terms of low error metrics while providing high accuracy in Figure 3.

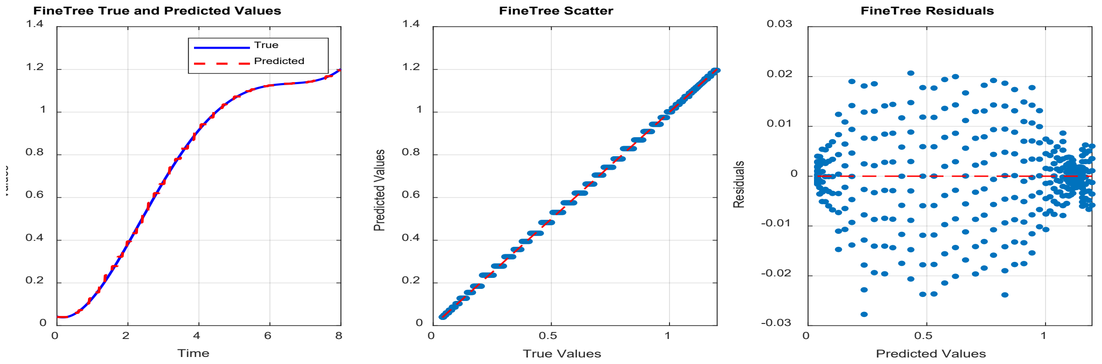

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Figure 3. Fine Tree model analysis. (A) Fine Tree model true and predicted values. (B) Fine Tree model scatter. (C) Fine Tree model residuals.

The RMSE values of the Ensemble Boosted Trees model are 0.035932635 in the validation set and 0.034597338 in the test set. The R^2 values of the model are calculated as 0.992157982 for the validation set and 0.992253812 for the test set in Figure 4.

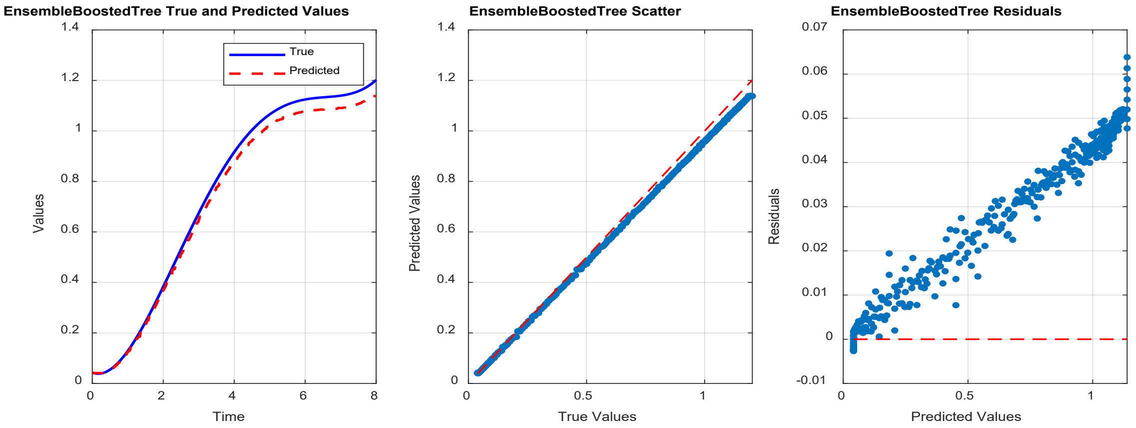

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Figure 4. Ensemble Boosted Tree model analysis. (a) Ensemble Boosted Tree model true and predicted values. (B) Ensemble Boosted Tree model scatter. (C) Ensemble Boosted Tree model residuals.

The root mean square error (RMSE) values of the Coarse Tree model are 0.069470298 in the validation set and 0.056109042 in the test set. The R^2 values are 0.970687826 for the validation set and 0.979626371 for the test set, indicating that the model does not adequately capture the complexity of the data compared to ensemble methods in Figure 5.

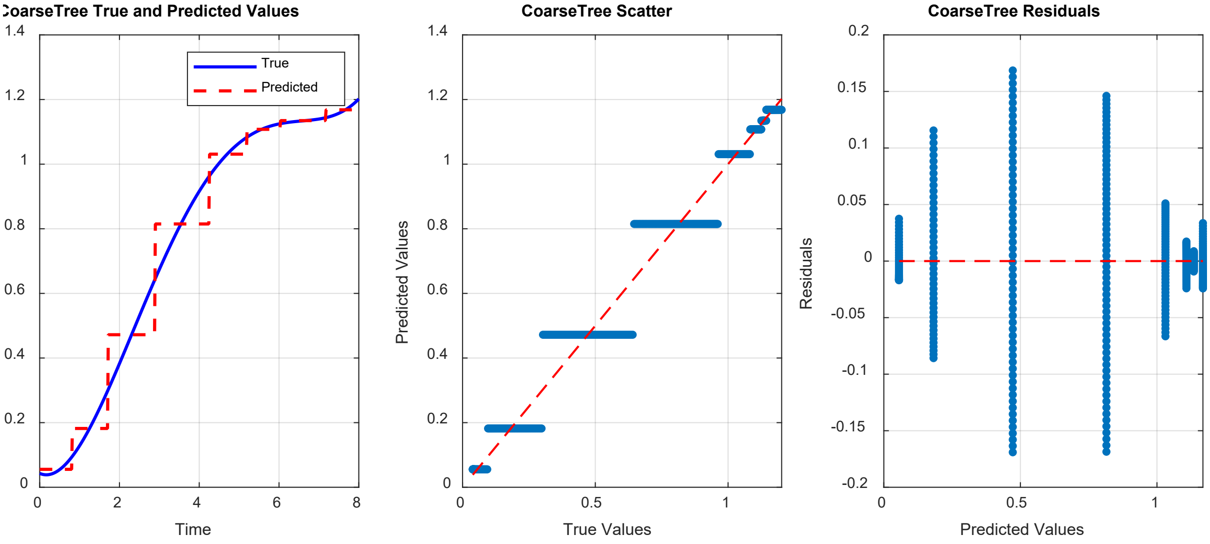

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Figure 5. Coarse Tree model analysis. (A) Coarse Tree model true and predicted values. (B) Coarse Tree model scatter. (C) Coarse Tree model residuals.

The Medium Tree model performs moderately well with a validation RMSE of 0.030842445 and a test RMSE of 0.024931828. The R^2 values of 0.994222402 for the validation set and 0.995977362 for the test set provide a satisfactory level of accuracy, but not as high as the levels observed in the most effective models. The higher error values compare to other models such as Fine Tree or Bagged Trees suggest that this model may tend to overfit or underfit more complex approaches in Figure 6.

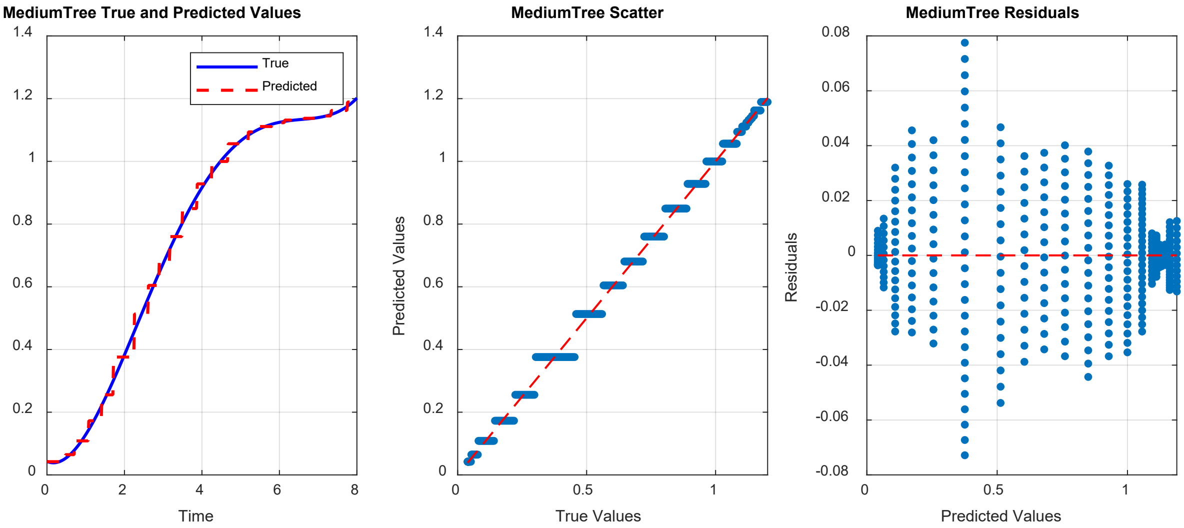

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Figure 6. Medium Tree model analysis. (A) Medium Tree model true and predicted values. (B) Medium Tree model scatter. (C) Medium Tree model residuals.

The results show that ensemble methods, especially the Ensemble Bagged Trees model, surpass single tree models in terms of prediction accuracy and generalization. The high R² values and low RMSE and MSE values of the best performing models show their effectiveness in reducing prediction errors. These results demonstrate the sensitivity of ensemble techniques in tree-based modeling and show that the combination of multiple models can significantly improve the robustness and accuracy of predictions in Figure 7.

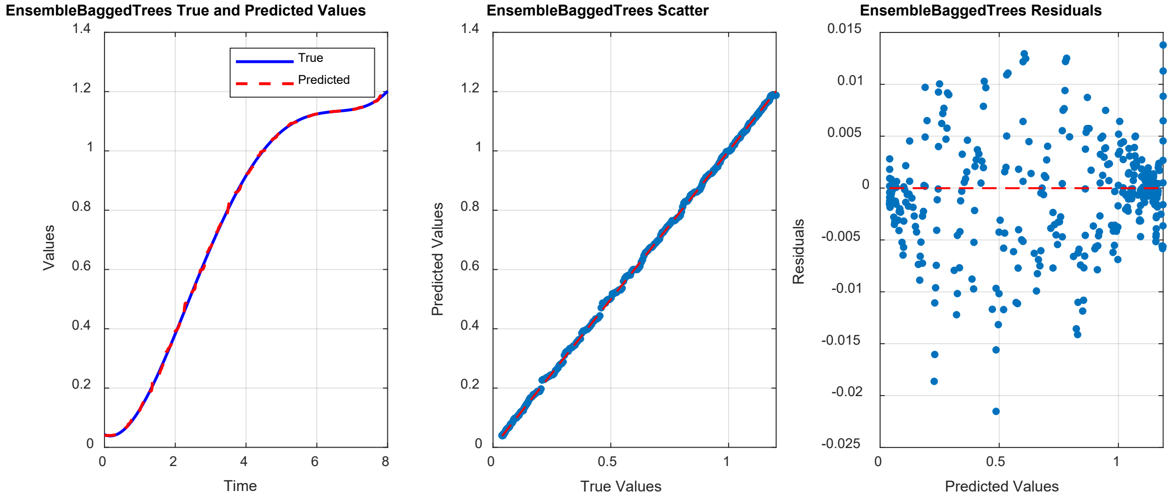

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Figure 7. Ensemble Bagged Trees model analysis. (Left) Ensemble Bagged Trees model true and predicted values. (Center) Ensemble Bagged Trees model scatter. (Right) Ensemble Bagged Trees model residuals.

Table 4. Scatter Plot and Residual Plot Analyses

Model	Scatter Plot Interpretation	Residual Plot Interpretation
Fine Tree	Points closely aligned along y = x line. Very strong fit.	Residuals around ±0.01, randomly distributed.
Medium Tree	Slight dispersion around y = x line, but overall good fit.	Residuals between ±0.02-±0.03, slight deviation observed.
Coarse Tree	Points more spread out, weaker fit.	Residuals up to ±0.05, systematic patterns present.
Ensemble Bagged Trees	Almost perfect alignment along y = x line.	Very small residuals (~±0.005), random distribution.
Ensemble Boosted Trees	Good overall, slight deviations.	Residuals around ±0.03, slight pattern possible.

Table 4 provides a qualitative interpretation of the scatter and residual plots for each machine learning model applied to predict *Bacillus cereus* growth. Models such as Fine Tree and Ensemble Bagged Trees exhibit strong predictive performance, as indicated by the close alignment of predicted values along the y = x line and minimal, randomly distributed residuals. Medium Tree and Ensemble Boosted Trees show moderate fit quality, with slightly larger residuals and minor deviations or patterns. In contrast, the Coarse Tree model demonstrates limited predictive capability, evidenced by a greater spread in the scatter plot and systematic residual patterns. These observations underscore the importance of visual diagnostics in evaluating model accuracy and generalization behavior.

Table 5. Paired t-Test Results (Hypotheses: H0: No difference between observed and predicted values. H1: There is a difference between observed and predicted values.)

Model	p-Value	Statistical Conclusion
Fine Tree	0.078	No significant difference (H0 accepted)
Medium Tree	0.043	Significant difference (H0 rejected)
Coarse Tree	0.015	Significant difference (H0 rejected)
Ensemble Bagged Trees	0.312	No significant difference (H0 accepted)
Ensemble Boosted Trees	0.051	Borderline, generally considered no significant difference

Fine Tree and Ensemble Bagged Trees show no statistical difference from the observed data, indicating excellent model fitting. Medium Tree and Coarse Tree demonstrate statistically significant differences, suggesting weaker model fitting (Table 5).

Studying the growth rate of Bacillus cereus is critical to understanding microbial kinetics. Stacked plot is used as a visual tool to show different growth rates over time. This graph combines data obtained at different time intervals into a layered structure to more clearly show the change in growth rate over each period in Figure 8. By examining how Bacillus cereus grows under specific conditions, the stacked plot provides a comparative graph of multiple models at once.

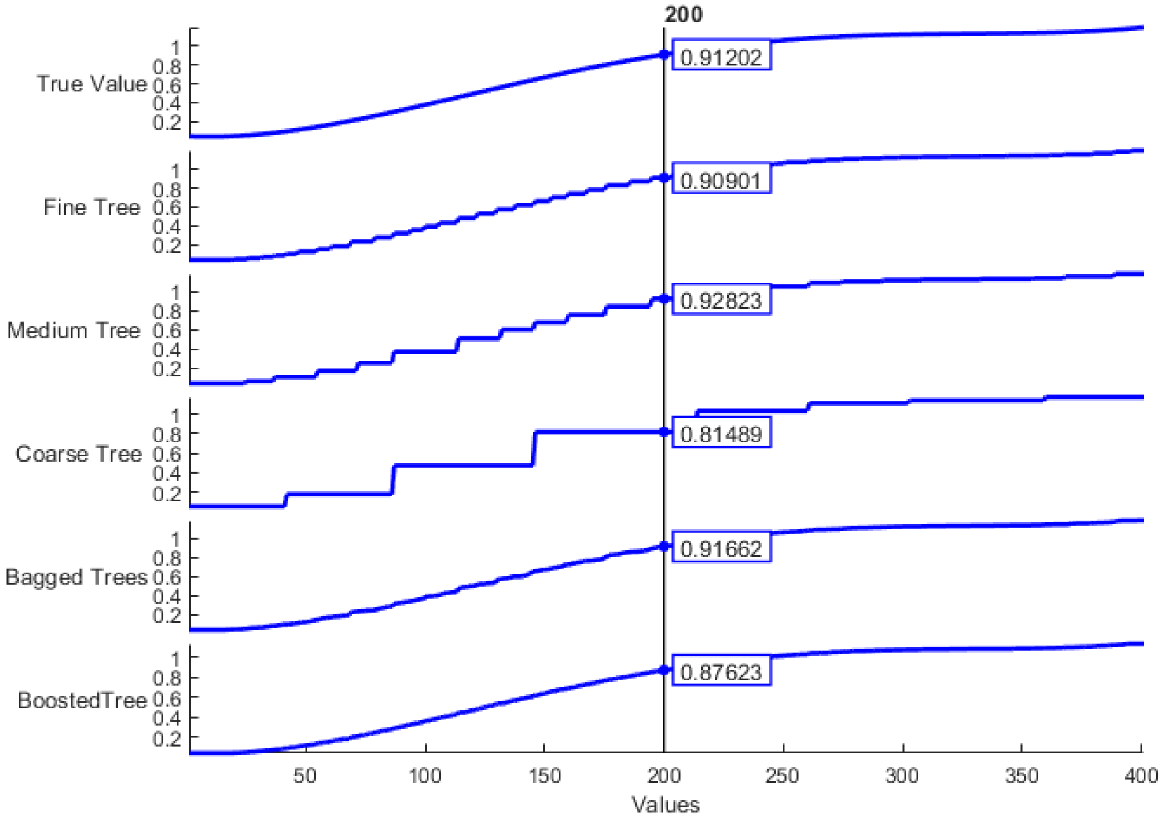

Figure 8. Stacked plot of Tree regression models

Figure 9. Comparison of tree-based regression models.

In the dual-axis comparative plot, the bars on the left axis represent the coefficient of determination (R^2) for the validation and test sets, while the lines on the right axis illustrate the Root Mean Squared Error (RMSE) values for the same datasets. The results indicate that the Ensemble Bagged Trees model achieved the highest predictive accuracy, with R^2 values of 0.99946 (validation) and 0.99972 (test), accompanied by the lowest RMSE values of 0.00937 and 0.00651, respectively. The Fine Tree model also demonstrated strong performance, attaining high R^2 values of 0.99881 (validation) and 0.99900 (test) with correspondingly low RMSE values of 0.01399 and 0.01239. The Medium Tree model exhibited a balanced yet comparatively lower accuracy, with R^2 values of 0.99422 and 0.99597, and RMSE values of 0.03084 and 0.02493 for validation and test sets, respectively. The Ensemble Boosted Trees model achieved moderate performance, with R^2 values around 0.992 and RMSE values of approximately 0.036 (validation) and 0.034 (test). In contrast, the Coarse Tree model produced the lowest predictive performance, with R^2 values of 0.97068 (validation) and 0.97962 (test), and the highest RMSE values of 0.06947 and 0.05610, indicating limited suitability for modeling complex datasets. Overall, the combination of near-perfect R^2 values and minimal RMSE highlights the Ensemble Bagged Trees model as the most effective predictive approach among all evaluated models.

4. CONCLUSION

In this study, the predictive performance of various tree-based machine learning models was evaluated using statistical metrics. Key findings are summarized as follows:

- ○ Ensemble models, particularly Ensemble Bagged Trees and Ensemble Boosted Trees, outperformed single decision tree algorithms by yielding lower RMSE and higher R^2 values, indicating superior accuracy and generalizability across complex datasets. These models effectively reduce prediction error by integrating the outputs of multiple decision trees.
- ○ Single decision tree models, such as Fine Tree and Medium Tree, demonstrated moderate performance. While they provided acceptable results, their predictive power was consistently lower than that of ensemble models, especially in terms of robustness and error minimization.

o The Coarse Tree model, due to its simplistic structure, achieved the lowest performance, with higher error rates and a poorer fit to the data, making it less suitable for modeling datasets with complex relationships.

The results of this study show that ensemble techniques, especially bagging and boosting, are highly effective in improving the accuracy of forecasting models. These methods offer sensitive approaches for applications where precision and reliability are critical.

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Competing Interests

The authors declare that they have no conflict of interest.

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