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Primary Mathematical Modeling of Growth on Phenol by *Pseudomonas* sp. strain Neni-4

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ABSTRACT

Primary modeling of microbial growth is essential for determining key parameters such as the maximum specific growth rate (μ_m), which are foundational for secondary modeling. Models such as the modified Gompertz, modified Logistic, modified Richards, Buchanan-3-phase, Baranyi-Roberts, modified Schnute, von Bertalanffy, and Morgan-Mercer-Flodin (MMF) models elucidate the impact of substrates on bacterial growth and biotransformation processes, which are vital for biotechnological applications such as wastewater treatment and bioremediation. In this study, the growth of a previously isolated phenol-degrading *Pseudomonas* sp. strain Neni-4 on phenol was modeled using the aforementioned primary models. Experimental data indicated that phenol concentrations ranging from 500 to 2500 mg/L were toxic, slowing bacterial growth and increasing lag periods from 5 to 7 hours. Among the primary models tested, the Huang model provided the best fit, evidenced by a high adjusted coefficient of determination, low RMSE, and AICc values, and favorable accuracy (AF) and bias factors (BF). The robustness of the Huang model highlights its suitability for modeling bacterial growth under toxic conditions, providing valuable insights for optimizing biotechnological processes that involve bacterial adaptation and growth under stress conditions. This model's ability to accurately describe the growth kinetics under such challenging conditions makes it a reliable tool for further bioprocess optimization and environmental applications.

INTRODUCTION

The chemical compound known as phenol emerges from industrial operations, including oil refining and petrochemical production, as well as in the manufacturing of compounds like phenolic resin, pigments, dyestuffs, paints, pharmaceutical and coal processing, and electronics manufacturing. Phenol is a toxic substance that contains a benzene ring structure with a hydroxyl group. Phenol toxicity stems from its ability to disrupt cellular membranes in acute toxicity and in long-term chronic toxicity by causing oxidative stress, mitochondrial dysfunction, immunotoxicity, genotoxicity, and DNA damage, to name a few [1]. The environmental buildup of phenol becomes severe because this compound resists natural microbial breakdown processes, as it is toxic to most microbes. Thus, phenol poses substantial health risks and ecological threats to soil and river ecosystems, as well as underground water sources. Industrial facilities remove phenol from wastewater by implementing physical extraction, chemical oxidation, and activated carbon adsorption methods. These methods show effectiveness, but they come with high costs and generate additional environmental contamination. The breakdown of phenol through bacterial or microbial processes represents a cost-efficient and environmentally beneficial method especially when pollution is very dilute or in soils. Microorganisms utilize phenol as their energy source to transform it into harmless by-products through their biological metabolic activities.

The release of phenol into the environment occurs through both industrial accidents and unintentional spills. The Indonesian tanker MV Endah Lestari, which capsized in 2001, resulted in the release of 600 tonnes of phenol and 18 tonnes of fuel into the ocean. The accident resulted in extensive pollution, which led to the death of marine life in 85 offshore fish cages [2]. Phenol and its compounds are hazardous to humans and other organisms, causing irritation to mucous membranes, skin, eyes, and the respiratory tract. Prolonged skin contact can lead to third-degree burns, and long-term exposure can result in liver and kidney damage [1]. Their toxicity is due to hydrophobicity and the production of phenoxyl radicals [3]. Phenol pollution is a

significant environmental issue, exacerbated by coal mining activities in Sumatra [4]. Primary models can accurately fit the sigmoidal characteristics of bacterial growth curves, including the lag, log (exponential), and stationary phases. This understanding facilitates the prediction of bacterial responses to environmental changes and nutrient availability. Establishing bacterial growth under control, non-inhibitory conditions is essential prior to investigating the effects of inhibitors, as this baseline facilitates comparative analysis in secondary modeling. Primary models elucidate growth under non-stressful conditions, while secondary models can forecast the impact of inhibitors on growth kinetics. The integration of primary and secondary models establishes a framework that improves the prediction and manipulation of microbial behavior in biotechnological applications. Primary models serve as essential frameworks in microbial kinetics, offering critical parameters and insights into bacterial growth in controlled environments. The parameters are essential for secondary models addressing substrate inhibition, which is crucial for thorough bioprocess optimization. Consequently, the integration of primary and secondary models provides a comprehensive framework for analyzing and influencing microbial growth across diverse industrial and environmental contexts.

In wastewater treatment, it is essential to understand the specific growth rate (μm), lag phase duration, and maximum population density through primary models. These parameters optimize conditions to enhance bacterial degradation of contaminants. In bioremediation, understanding bacterial growth and responses to vary pollutant concentrations is essential for formulating effective environmental cleanup strategies. Primary models such as the modified Gompertz, modified Logistic, modified Richards, Baranyi-Roberts, and modified Schnute offer essential data for these applications. Secondary models developed by Haldane, Andrews, Yano, and Aiba build upon foundational data to incorporate inhibitory effects, thereby enhancing the understanding of microbial kinetics across diverse conditions. This approach is essential for optimizing biotechnological processes to enhance performance and efficiency [5–13]. Previously, we have isolated several phenol-degrading bacteria from Indonesian soils [14–16]. This study reports the isolation of a novel and more efficient bacterial strain capable of degrading phenol at a significantly faster rate than many phenol-degrading strains reported to date. We intend to utilize these strains in future local remediation of phenol-contaminated soils, and the isolation of local phenol-degraders is vital, as importing foreign, nonindigenous phenol-degraders might cause unwanted ecological issues in the near future [17]. To support its biotechnological potential, growth modeling was carried out using several well-established kinetic models, including the modified Gompertz, modified Logistic, modified Richards, Baranyi-Roberts, von Bertalanffy, MMF, Huang, and modified Schnute models. The objective is to determine the most suitable model for accurately describing the growth dynamics of this high-performing strain, thereby enhancing predictive capabilities and informing the optimization of phenol biodegradation processes.

MATERIALS AND METHODS

Phenol-degrading bacterium growth medium

This bacterium was previously isolated as a molybdenum reducer [18]. The growth of this bacterium on phenol was carried out according to [15]. An aliquot of 0.1 mL from a freshly cultured overnight suspension of the bacterium in nutrient broth was transferred to 100 mL of medium contained within a 250 mL volumetric flask. The growth medium used was Minimal Salt Medium (MSM), which included phenol at various concentrations from 550 to 2250 mg/L as the only carbon source and (g/L) 0.50 NH_4NO_3, 0.50 KH_2PO_4, 0.50 $MgSO_4 \cdot 7H_2O$, 0.10 $CaCl_2$, 0.50 K_2HPO_4, 0.20 NaCl and 0.01 $MnSO_4 \cdot 7H_2O$, 0.01 $FeSO_4 \cdot 7H_2O$ [4]. The pH of this medium was adjusted to pH 7.0. For sterilization purposes, PTFE syringe filters (0.45 micron) were employed. This culture was then incubated at 25°C on a shaking incubator (Certomat R, USA) set to 150 rpm, continuing for a period of 48 h. One mL samples from the bacterial culture were serially diluted using sterile tap water for subsequent enumeration of colony-forming units per milliliter (CFU/mL) and then converted into biomass (mg) according to standard method [19].

Fitting of the data

Nonlinear regression, utilizing the Marquardt method, was employed to fit growth data to nonlinear equations (Table 1), aiming to minimize the sum of squared residuals. This study utilized CurveExpert Professional software (Version 1.6). This iterative method achieves the best fit by minimizing the discrepancy between predicted and observed values. The program facilitates both manual and automated input of initial parameter estimates. A four-data point steepest ascent search yielded the maximum specific growth rate (μ_m) for Mo-blue production. The x-axis intercept of the projected line from the steepest ascent was utilized to determine the duration of the lag phase (l). The final data point indicating the plateau period facilitated the estimation of the asymptotic value (A).

Table 1. Growth models used in this study.

Model	p	Equation
Modified Logistic	3	$y = \frac{A}{1 + exp\left[\frac{4\mu_m}{A}(\lambda - t) + 2\right]}$
Modified Gompertz	3	$y = Aexp\left\{-exp\left[\frac{\mu_m \cdot e}{A}(\lambda - t) + 1\right]\right\}$
Modified Richards	4	$y = A\left\{1 + vexp(1+v)exp\left[\frac{\mu_m}{A}(1+v)\left(1+\frac{1}{v}\right)(\lambda - t)\right]\right\}^{\left(\frac{-1}{v}\right)}$
Modified Schnute	4	$y = \left(\mu_m \frac{(1-\beta)}{\alpha}\right)\left[\frac{1-\beta exp(\alpha\lambda + 1 - \beta - \alpha t)}{1-\beta}\right]^{\frac{1}{\beta}}$
Baranyi-Roberts	4	$y = N_0 + \mu_m t + \frac{1}{\mu_m} ln(e^{-\mu_m t} + e^{-h_0} - e^{-\mu_m t - h_0}) - ln\left[1 + \frac{e^{\mu_m t + \frac{1}{\mu_m} ln(e^{-\mu_m t} + e^{-h_0} - e^{-\mu_m t - h_0})}}{e^{(A - N_0)}}\right]$
Von Bertalanffy	3	$y = k\left[1 - \left[1 - \left(\frac{A}{k}\right)^3\right] exp^{-\left(\frac{\mu_m t}{3k}\right)^{\frac{1}{3}}}\right]$
Huang	4	$y = A + \mu_m - ln\left(e^A + (e^{\mu_m} - e^A)e^{-\mu_m B(t)}\right)$ $B(t) = t + \frac{1}{\alpha} ln\frac{1 + e^{-\alpha(t-\lambda)}}{1 + e^{\alpha\lambda}}$
Buchanan Three-phase linear model	3	$Y = N_0$, IF $X < LAG$ $Y = N_0 + K(X - \lambda)$, IF $\lambda \leq X \geq X_{MAX}$ $Y = A$, IF $X \geq X_{MAX}$
Morgan-Mercer-Flodin (MMF)	4	$y = A - \frac{(A - \beta)}{1 + (\mu_m t)^{\delta}}$

Note:
A= Microorganism growth upper asymptote;
N_0= Microorganism growth lower asymptote;
u_m= maximum specific microorganism growth rate;
v= affects near which asymptote maximum growth occurs.
λ=lag time
e = exponent (2.718281828)
t = sampling time
α, β, k, δ= curve fitting parameters
h_0 = a dimensionless parameter quantifying the initial physiological state of the reduction process. For the Baranyi-Roberts model, the lag time (λ) (h^{-1}) or (d^{-1}) can be calculated as $h_0 = \mu_m$

For modified Schnute, $A = m/a$

Statistical analysis

The following tests or statistical discrimination or error functions include Bias Factor (BF), Accuracy Factor (AF) [20], root-mean-squared error (RMSE), adjusted coefficient of determination (R^2) [21], and corrected Akaike Information Criterion (AICc) [22,23]. In general, n is the total number of observations, *Obi* and *Pdi* are the predicted and observed values, and p is the total number of parameters of the model [24].

RMSE was calculated using the following formula;

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}(Pd_i - Ob_i)^2}{n-p}} \qquad \text{(Eqn. 1)}$$

BF and AF were calculated using the following formula;

$$Bias\ factor = 10\left(\sum_{i=1}^{n} log\frac{(Pd_i/Ob_i)}{n}\right) \qquad \text{(Eqn. 2)}$$

$$Accuracy\ factor = 10\left(\sum_{i=1}^{n} log\frac{|(Pd_i/Ob_i)|}{n}\right) \qquad \text{(Eqn. 3)}$$

AICc was calculated using the following formula;

$$AICc = 2p + n\ln\left(\frac{RSS}{n}\right) + \frac{2(p+1)+2(p+2)}{n-p-2} \qquad \text{(Eqn. 4)}$$

Adjusted coefficient of determination (R^2) was calculated using the following formula;

$$Adjusted\ (R^2) = 1 - \frac{RMS}{S_Y^2} \qquad \text{(Eqn. 5)}$$

$$Adjusted\ (R^2) = 1 - \frac{(1-R^2)(n-1)}{(n-p-1)} \qquad \text{(Eqn. 6)}$$

RESULTS AND DISCUSSION

The growth of the bacterium on phenol

Phenol-degrading bacteria are optimal for phenol remediation based on economic considerations. The Biodegradation of phenol by microorganisms has been the subject of extensive global research. *Pseudomonas* species are the main degraders [25–28], *Bacillus* spp. [29–35], *Alcaligenes* sp. [36], *Ochrobactrum* sp. [37], *Acinetobacter* sp. [38,39] and *Rhodococcus* species [40–46] are also reported. Each degrader possesses distinct properties, including tolerance to high concentrations of phenol, salinity, and heavy metals, as well as the capacity to thrive at extreme pH levels or temperatures. The presence of numerous bacteria capable of phenol degradation renders bioremediation a more effective method for this process. Currently, a limited number of primary models have been employed. The growth of *Pseudomonas* sp. strain Neni-4 on different concentrations of phenol was initially transformed into natural logarithm (**Fig. 1**) prior to modeling.

Bacterial growth on phenol often exhibits a unique phase where the specific growth rate starts at zero and gradually accelerates to a maximal value (μ_{max}), resulting in a lag time (λ) [47]. The sigmoidal shape observed in bacterial growth curves includes a lag phase, wherein bacterial cells adjust their growth processes to new environmental conditions following a period of dormancy, especially during storage.

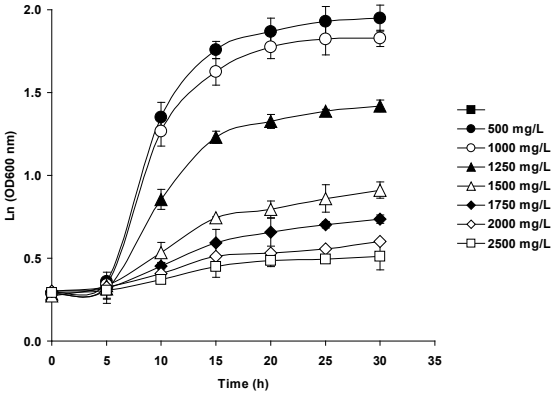

Fig. 1. The growth of *Pseudomonas* sp. strain Neni-4 on various concentrations of phenol.

The preparatory phase, referred to as the "lag period," involves cellular adjustments to new conditions before the onset of exponential growth. Baranyi and Roberts characterized this phase as a transitional period connecting two independent growth systems. The authors argued that incorporating lag time or a parameter in growth models is primarily for convenience rather than offering a mechanistic explanation. This method facilitates modeling and understanding bacterial growth patterns under various conditions [48].

The values obtained, especially the maximum specific growth rate (μ_m), are essential for later phases in secondary modeling. The parameters are essential as they offer foundational insights required for precise modeling of microbial behavior across diverse environmental conditions and stresses. Subsequent analyses often utilize secondary models developed by Monod, Haldane, Aiba, and Teissier to clarify the influence of substrates on bacterial growth and the transformation rates of xenobiotics. These models are crucial for understanding the influence of varying substrate concentrations on microbial growth kinetics and biotransformation processes, which are essential in biotechnological applications such as wastewater treatment, bioremediation, and biochemical production [49,50].

Multiple primary models (**Figs. 2-10**) were employed to analyze the growth rate, with the majority demonstrating visually satisfactory fits. The Huang model emerged as the most effective based on statistical analysis, exhibiting the highest adjusted coefficient of determination and the lowest RMSE values. Additionally, accuracy and bias factors fell within the optimal range, with the exception of the AICc function (Table 2). Modeling results demonstrate that phenol concentrations of 1000 mg/L and above, when used as the sole carbon source, exhibit toxicity, inhibiting bacterial growth at elevated levels. This toxicity leads to an extension of lag periods, varying from 5 to 7 hours (**Fig. 11**). Phenol concentrations exceeding 2500 mg/L are impractical because of the elevated vaporization rate at these levels, along with the potential toxicity of the vaporized phenol to researchers. The experimental data obtained indicate that phenol exhibits toxicity and inhibits growth rates at elevated concentrations, consistent with findings from nearly all existing studies on phenol biodegradation. The Huang model illustrates the growth of the bacterium at different phenol concentrations (**Fig. 11**) and its impact on the specific growth rate is presented in **Fig. 12**.

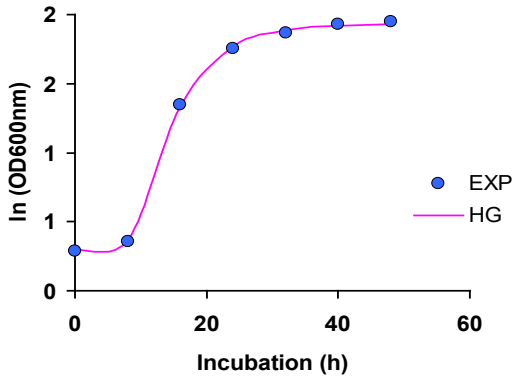

Fig. 2. Growth curve fitting of the *Pseudomonas* sp. strain Neni-4 on 1000 mg/L phenol using the Huang model.

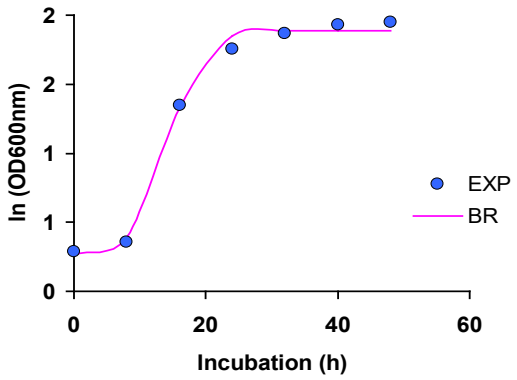

Fig. 3. Growth curve fitting of the *Pseudomonas* sp. strain Neni-4 on 1000 mg/L phenol using the Baranyi-Roberts model.

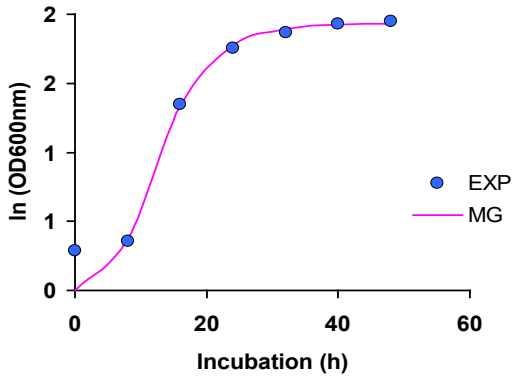

Fig. 4. Growth curve fitting of the *Pseudomonas* sp. strain Neni-4 on 1000 mg/L phenol using the modified Gompertz model.

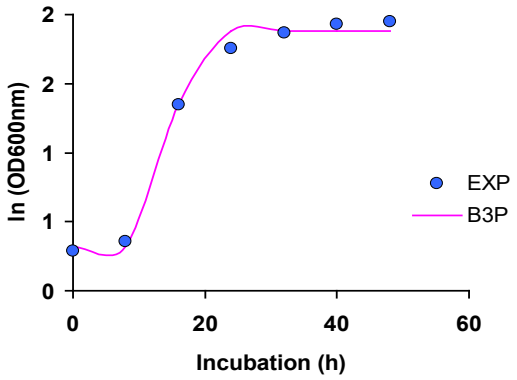

Fig. 5. Growth curve fitting of the *Pseudomonas* sp. strain Neni-4 on 1000 mg/L phenol using the Buchanan-3-phase model.

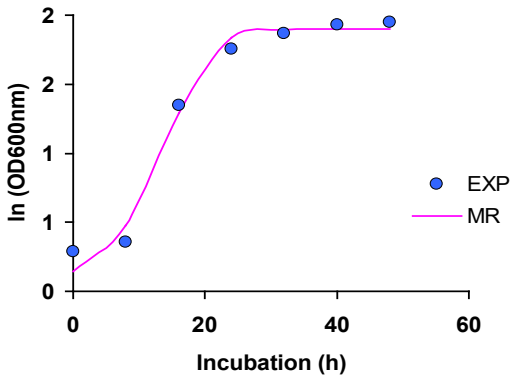

Fig. 6. Growth curve fitting of the *Pseudomonas* sp. strain Neni-4 on 1000 mg/L phenol using the modified Richards model.

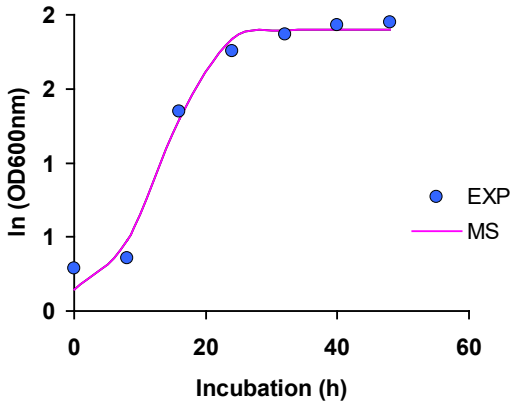

Fig. 7. Growth curve fitting of the *Pseudomonas* sp. strain Neni-4 on 1000 mg/L phenol using the modified Schnute model.

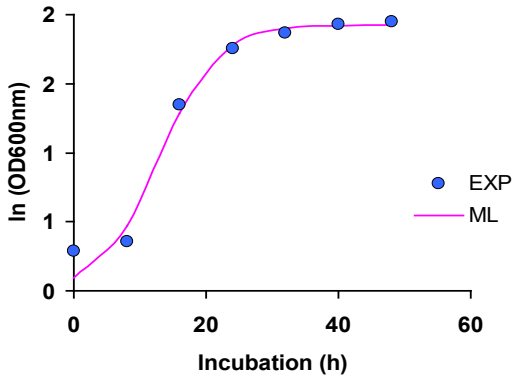

Fig. 8. Growth curve fitting of the *Pseudomonas* sp. strain Neni-4 on 1000 mg/L phenol using the modified Logistics model.

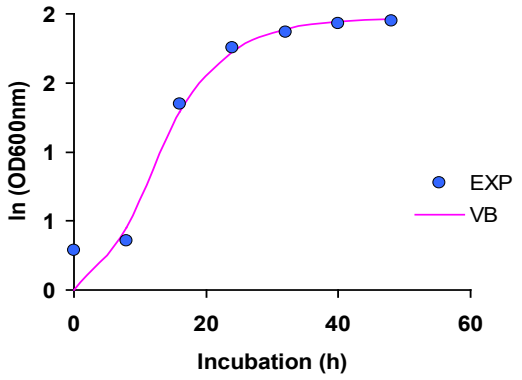

Fig. 9. Growth curve fitting of the *Pseudomonas* sp. strain Neni-4 on 1000 mg/L phenol using the von Bertalanffy model.

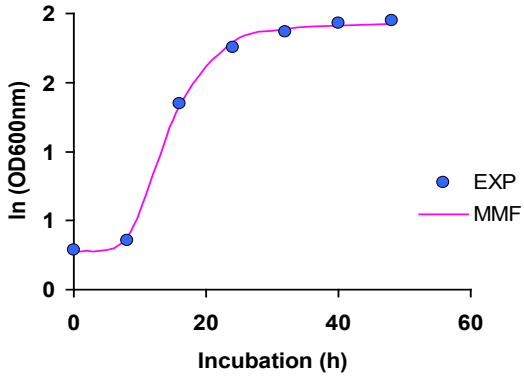

Fig. 10. Growth curve fitting of the *Pseudomonas* sp. strain Neni-4 on 1000 mg/L phenol using the MMF model.

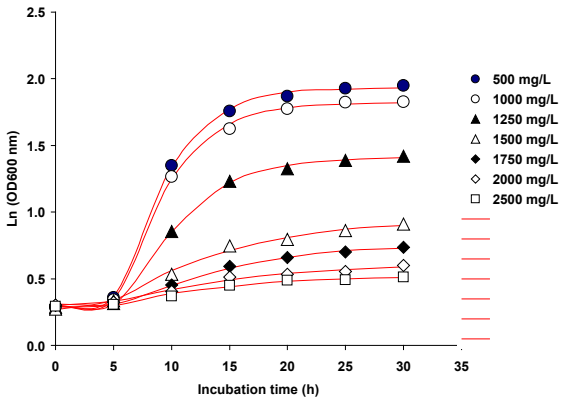

Fig. 11. Fitting the growth rate of *Pseudomonas* sp. strain Neni-4 at various phenol concentrations using the Huang model (red lines).

Table 2. Statistical analysis of the growth models.

Model	p	RMSE	adR²	AF	BF	AICc
Huang	4	0.024	0.998	1.012	1.003	19.697
Baranyi-Roberts	4	0.073	0.985	1.042	1.001	35.452
modified Gompertz	3	0.142	0.959	2.165	0.471	2.764
Buchanan-3-phase	3	0.079	0.985	1.051	1.000	-5.456
modified Richards	4	0.126	0.957	1.172	0.940	43.123
modified Schnute	4	0.090	0.978	1.172	0.940	38.377
modified Logistics	3	0.119	0.967	1.236	0.883	0.325
von Bertalanffy	3	0.157	0.948	3.269	0.329	4.119
MMF	4	0.038	0.996	1.031	0.999	26.261

Note:
p parameter
RMSE Root Mean Square Error
R^2 Coefficient of Determination
adR^2 Adjusted Coefficient of Determination
AICC Corrected Akaike Information Criterion
BF Bias Factor
AF Accuracy Factor

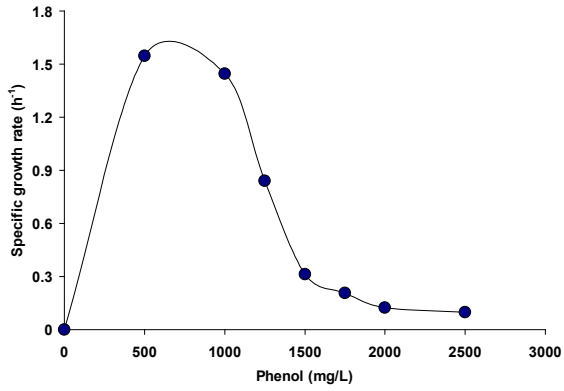

Fig. 12. The effec of phenol concentrations on the specific growth rate of *Pseudomonas* sp. strain Neni-4 on phenol as modelled using the Huang model.

Accurate modeling of bacterial growth and substrate inhibitory effects in microbial kinetics is crucial for optimizing bioprocesses, ensuring product safety, and enhancing the understanding of microbial ecology. Key models, such as the modified Gompertz, modified Logistic, modified Richards, Baranyi-Roberts, modified Schnute, von Bertalanffy, and Morgan-Mercer-Flodin (MMF) models, are essential in this context. The models characterize bacterial growth in non-inhibitory environments, quantifying essential parameters including specific growth rate (μ_m), lag phase duration, and maximum population density. Comprehending these parameters is essential for progressing to more intricate secondary modeling, which integrates inhibitory effects through models such as Haldane, Andrews, Yano, and Aiba. The primary models are crucial for determining key growth parameters in microbiology and biochemical engineering, specifically for defining the replication speed of bacteria under specific conditions.

These models provide detailed insights into bacterial growth dynamics, enabling researchers to predict bacterial responses to environmental changes and variations in nutrient availability. This is essential for applications including wastewater treatment, bioremediation, and the production of biofuels and other bioproducts [51–55]. Lihan Huang developed the Huang model in 2013, which constitutes a significant step forward in the predictive mathematical modeling of bacterial growth in the food industry. However, it is now being used to study the effect of stresses, especially when bacteria are grown in the presence of xenobiotics, which are industrial chemicals not found in biological systems. The Huang model differs from classical models, such as the Gompertz or logistic equations, in that it utilizes biologically interpretable parameters, which enhance its predictive capabilities, particularly in complex scenarios like polluted soils or industrial waste streams. The Huang model is designed to better capture the lag phase, exponential growth, and stationary phase of bacterial populations.

The model is a sigmoidal function based on first-order kinetics, but its parameters are based on real biological processes such as lag time (λ), maximum specific growth rate (μ_{max}), and the population density that approaches zero (A). this model is easier to compute and fits real-world data, making it straightforward to integrate with software tools commonly used in microbiological and environmental research. huang's method is especially helpful currently for modeling bacteria that thrive in toxic environments, such as soils or water bodies exposed to or contaminated by pesticides, pharmaceuticals, or industrial solvents. it is crucial to understand how bacteria respond to xenobiotics in order to monitor and clean up the environment effectively. when bacteria come into contact with these types of foreign substances, their growth is often slowed down, stopped, or altered in ways that are difficult to predict. because they make static assumptions and can't handle nonlinear dynamics caused by chemical stressors, traditional growth models don't take this complexity into account.

The Huang model can handle this kind of variability by allowing the lag phase and growth rate to change based on the findings of this study, where only the Huang model successfully modeled the lag phase well. The model is also sensitive to the size of the initial inoculum and various environmental factors, such as pH and temperature, providing a comprehensive understanding of how microbes respond. This makes it perfect for simulating bioprocesses in the real world. Examples of the Huang model's applications in modeling growth on xenobiotics are beginning to be recognized [56–58]. One of its best features is the Huang model's biological relevance, as each parameter has a direct biological meaning. This makes it easier to use for testing hypotheses and designing experiments. The model is resistant to outliers and performs better with noisy data than many traditional models, including the modified Gompertz model and the modified logistic model.

Coefficients obtained from model-fitting exercises are biologically relevant parameters used in subsequent modeling efforts. Mechanistic models are essential in fundamental research, as they improve our understanding of the physical, chemical, and biological processes underlying observed growth profiles. Mechanistic models exhibit greater efficacy under constant conditions, as they elucidate the fundamental processes that govern observed patterns. This foundation closely resembles biological systems, rendering these models especially effective and dependable for extrapolating beyond initially observed conditions. Three-parameter models offer simplicity and ease of use, enabling quicker understanding and implementation. Their stability, resulting from less correlated parameters, ensures consistent results across various datasets [59]. These models, having fewer parameters to estimate, offer increased degrees of freedom, which enhances the accuracy and reliability of parameter estimation, particularly in small datasets. The biological interpretation of model parameters guarantees relevance and accuracy in depicting biological phenomena, thereby increasing their utility in scientific research. Mechanistic models enable researchers to analyze complex biological processes, leading to discoveries that contribute to practical applications in biotechnology, medicine, and environmental science. They are effective for predictive modeling as they closely replicate biological systems, rendering them valuable tools for scientific research and practical applications [60].

CONCLUSION

The study of bacterial growth on phenol reveals a phase in which the specific growth rate initially begins at zero and then progressively increases to a maximum value, indicating a distinct lag period. This phase serves as a preparatory adjustment period for bacterial cells and is essential for comprehending bacterial adaptation to new environmental conditions. The primary modeling of microbial growth is crucial for identifying key growth parameters, such as the maximum specific growth rate (μ_m), and offers essential insights for subsequent modeling efforts. These insights are essential for biotechnological applications, including wastewater treatment, bioremediation, and biochemical production. The experimental data, corroborated by multiple primary models, demonstrate that phenol exhibits toxicity and suppresses bacterial growth at elevated concentrations. The Huang model exhibited the optimal fit among the tested models, as indicated by statistical analysis, normality tests, and critical parameters including the adjusted coefficient of determination, RMSE, accuracy, and bias factors. The model parameters, particularly the value of μm, will be employed in future publications to model the inhibitory effect of phenol on the growth rate of this bacterium. The study provides valuable insights into microbial growth kinetics, which are essential for optimizing biotechnological processes that involve bacterial adaptation and growth under stress conditions.

AI DECLARATION

During manuscript preparation, AI tools such as ChatGPT (OpenAI), Grammarly, and QuillBot were used to assist with structure, clarity, grammar, and phrasing. All AI-assisted content was carefully reviewed and edited by the authors, who take full responsibility for the final version and its scientific integrity.

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