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Primary Mathematical Modeling of Growth on Phenol by Bacillus sp. Strain Neni-10

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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, Morgan-Mercer-Flodin (MMF), and Huang elucidate the impact of substrates on bacterial growth and biotransformation processes, vital for biotechnological applications like wastewater treatment and bioremediation. In this study, the growth of a previously isolated phenol-degrading Bacillus sp. strain Neni-10 on phenol was modeled using the aforementioned primary models. Experimental data indicated that phenol concentrations ranging from 250 to 2200 mg/L were toxic, slowing bacterial growth and increasing lag periods from 5.8 to 9.4 hours. Among the primary models tested, the modified Gompertz 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 modified Gompertz 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

Phenol, a pollutant generated in various industrial processes, is an aromatic compound with a benzene ring structure. It accumulates in soil, rivers, and groundwater due to artificial contamination, causing significant toxicity to both animals and plants. Its persistence in the environment is a major concern because it is not easily decomposed naturally. Major sources of phenol pollution include petroleum refining, the petrochemical industry, phenolic resin production, pharmaceutical companies, coal conversion plants, and electronics industry plants. Various physicochemical methods such as chemical oxidation, solvent extraction, and adsorption by activated carbon are used to remove phenol from wastewater. However, these methods can be costly and sometimes lead to secondary contamination. In contrast, the biological treatment of phenol using phenol-utilizing microorganisms is more economical and efficient. This approach

harnesses the natural metabolic pathways of microorganisms to degrade phenol, thereby minimizing the risk of secondary pollution. Accidents also contribute significantly to phenol pollution. For instance, the 2001 capsize of the Indonesian tanker MV Endah Lestari, which spilled 18 tonnes of fuel and 600 tonnes of phenol, resulted in severe contamination of coastal waters and the death of marine life in 85 offshore cages [1]. 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 thirddegree burns, and long-term exposure can result in liver and kidney damage [2]. 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 effectively capture the sigmoidal nature of bacterial growth curves, encompassing the lag, log (exponential), and stationary phases. This detailed understanding aids in predicting bacterial responses to environmental changes and nutrient availability. Establishing bacterial growth under controlled, non-inhibitory conditions is crucial before exploring the effects of inhibitors, as this baseline allows for comparative analysis in secondary modeling. Once primary models describe growth under non-stressful conditions, secondary models can predict how inhibitors affect growth kinetics. Together, primary and secondary models form an integrated framework that enhances our ability to predict and manipulate microbial behavior in biotechnological applications. Primary models are foundational in microbial kinetics as they provide the necessary parameters and insights into bacterial growth under controlled conditions. These parameters are critical for secondary models that focus on substrate inhibition, which is vital for comprehensive bioprocess optimization. Thus, primary and secondary models together offer a robust framework to understand and influence microbial growth in various industrial and environmental applications. For example, in wastewater treatment, understanding the specific growth rate (μ_m) , lag phase duration, and maximum population density through primary models is essential.

These parameters help optimize conditions to maximize bacterial degradation of contaminants. Similarly, in bioremediation, knowing how bacteria grow and respond to different concentrations of pollutants informs the development of effective strategies to clean up contaminated environments. Primary models like the modified Gompertz, modified Logistic, modified Richards, Baranyi-Roberts, and modified Schnute provide the foundational data required for these applications. Secondary models, such as those developed by Haldane, Andrews, Yano, and Aiba, then build on this foundational data to incorporate inhibitory effects, providing a more comprehensive understanding of microbial kinetics under various conditions. This comprehensive approach is crucial for fine-tuning biotechnological processes to achieve optimal performance and efficiency [5-13].

This research intends to create models for the growth of a bacterium on phenol, a toxic substance, using several main models like as the modified Gompertz, modified Logistic, modified Richards, Baranyi-Roberts, and modified Schnute models. The goal is to identify the best appropriate model for the growth curve to better understand bacterial growth in these conditions and improve the accuracy of predictions for enhancing biotechnological processes associated with phenol degradation.

MATERIALS AND METHODS

Phenol-degrading bacterium growth medium

This bacterium was previously isolated as a molybdenum reducer [14]. The growth of this bacterium on phenol was carried 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 NH4NO3, 0.50 KH2PO4, 0.50 MgSO4 · 7H2O, 0.10 CaCl₂, 0.50 K₂HPO₄, 0.20 NaCl and 0.01 MnSO₄·7H₂O, 0.01 FeSO₄·7H₂O [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 [16].

Fitting of the bacterial growth data on phenol

In this study, we utilized CurveExpert Professional (Version 1.6) software to model bacterial growth on phenol. This software minimizes the sum of squares of the differences between predicted and measured values using the Marquardt algorithm. The Marquardt algorithm is an iterative method that adjusts parameters to reduce the error between predicted and observed data, ensuring an optimal fit for the growth curve [17]. By applying this method, we aimed to identify the most accurate primary model (Table 1) or describing bacterial growth under these conditions. The CurveExpert software's ability to handle nonlinear regression and its robust optimization capabilities made it an ideal choice for this study.

Table 1. Mathematical modeling of the growh of phenol by Bacillus sp. strain Neni-10

Model	р	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	$y = k \left[1 - \left[1 - \left(\frac{A}{k} \right)^3 \right] exp^{-\left(\frac{\mu_m t}{3k}\right)^3} \right]$
Huang	4	$y = A + \mu_m - ln(e^A + (e^{\mu_m} - e^A)e^{-\mu_m B(t)})$ $B(t) = t + \frac{1}{\alpha}ln\frac{1 + e^{-\alpha(t-\lambda)}}{1 + e^{\alpha\lambda}}$
Buchanan Three-phase linear model	3	$Y = N_{\theta}, \text{ IF } X < \text{LAG}$ $Y = N_{\theta} + K(X - \lambda), \text{ IF } \lambda \leq X \geq X_{MAX}$ $Y = \text{ A. IF } X > X_{MAX}$
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 $\alpha, \beta, k, \delta =$ curve fitting parameters

= 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, $A = \mu/\alpha$

The Marquardt algorithm, combining the principles of the Gauss-Newton algorithm and the method of gradient descent, iteratively refines the parameter estimates. This ensures that the

model closely aligns with the observed experimental data, providing a precise representation of the bacterial growth dynamics. By using CurveExpert Professional, we were able to compare various primary models such as the modified Gompertz, modified Logistic, modified Richards, Baranyi-Roberts, and modified Schnute. The goal was to determine which model best fits the experimental data, indicated by metrics like the adjusted coefficient of determination (R²), root mean square error (RMSE), Akaike Information Criterion corrected (AICc), accuracy factor (AF), and bias factor (BF).**Statistical analysis** Extensive error function analyses were utilized in this study and include Root-mean-square error (*RMSE*), and Ross's bias factor (BF), and accuracy factor (AF) and adjusted coefficient of determination ($adjR^2$) [18]. The rootmean-square error or RMSE was calculated according to Eq. 1;

The RMSE was calculated as follows,

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

where

n number of experimental data Pd_i predicted values by the model

 Ob_i experimental data

p parameters number of the model

 R^2 and $adjR^2$ (Eqns. 2 and 3) were calculated as follows;

Adjusted
$$(R^2) = 1 - \frac{RMS}{s_Y^2}$$
 (Eqn. 2)

Adjusted
$$(R^2) = 1 - \frac{(1 - R^2)(n - 1)}{(n - p - 1)}$$
 (Eqn. 3)

where

 s_y^2 is the total variance of the y-variable and RMS is the Residual Mean Square

The Akaike Information Criterion (AIC) is an informationtheoretic approach to model selection, emphasizing the minimization of AIC values to identify the optimal model. However, a lower AIC value is not always preferable; for instance, an AICc value of -10 is generally more favorable than -1. The AIC includes a penalty for increasing model complexity, discouraging overly complicated models. When dealing with a small number of parameters, researchers often use the corrected AIC (AICc), which provides more precise model comparisons by adjusting for small sample sizes [20]. AICc is calculated using the following equation (**Eqn. 4**);

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

Where

n number of data points

p parameter numbers of the model

Equations 5 and 6, known as Accuracy Factor (AF) and Bias Factor (BF), are metrics used to assess the goodness-of-fit of models commonly applied in predicting bacterial growth in food science [21].

The statistics determine a perfect connection between experimental and projected results. A fail-safe model has a Bias Factor (BF) beyond 1.0, whereas a fail-dangerous model has a BF below 1.0. The AF is consistently less than one, with values approaching one as projected by the most precise models.

Bias factor =
$$10^{\left(\sum_{i=1}^{n} \log \frac{(Pd_i / Ob_i)}{n}\right)}$$
 (Eqn. 5)
Accuracy factor = $10^{\left(\sum_{i=1}^{n} \log \frac{[(Pd_i / Ob_i)]}{n}\right)}$ (Eqn. 6)

RESULTS AND DISCUSSION

The growth of the bacterium on phenol

Phenol-degrading bacteria are ideal for phenol remediation due factors. Biodegradation of phenol by to economic microorganisms has long been an object of intense research globally. Bacteria that could degrade phenol include Pseudomonas species [22-25], Bacillus spp. [26-32], Alcaligenes sp. [33], Ochrobactrum sp. [34], Acinetobacter sp. [35,36] and Rhodococcus species [37-43]. Each of these degraders have its own unique properties such as the ability to tolerate high concentration of phenol, salt tolerant, heavy metals tolerant and the ability to grow at either extreme pHs or temperature. The existence of multitude of bacteria with phenoldegrading ability makes bioremediation the more ideal method for phenol degradation. To date very few primary models have been utilized. The growth of Bacillus sp. strain Neni-10 on various concentrations of phenol were first converted to natural logarithm (Fig. 1) before modelling.



Fig. 1. The growth of *Bacillus* sp. strain Neni-10 on various concentrations of phenol.

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) [44]. This sigmoidal shape in bacterial growth curves features a lag period, during which bacterial cells adapt their growth mechanisms to new environmental conditions after dormancy, particularly during storage. This preparatory phase, known as the "lag period," is when cells adjust to new conditions before entering exponential growth. Baranyi and Roberts described this phase as a transient period linking two autonomous growth systems.

They posited that the introduction of lag time or a parameter in growth models serves primarily for convenience rather than providing a mechanistic explanation. This approach helps in modeling and understanding the growth patterns of bacteria under varying conditions [45]. The values obtained, particularly the maximum specific growth rate (μ_m) , are invaluable for subsequent stages in secondary modeling. These parameters are crucial as they provide foundational insights necessary for accurately modeling microbial behavior under a variety of environmental conditions and stresses. In further analyses, secondary models such as those developed by Monod, Haldane, Aiba, and Teissier are frequently employed to elucidate the impact of substrates on bacterial growth or the transformation rates of xenobiotics. These models are instrumental in describing how different concentrations of substrates can influence microbial growth kinetics and biotransformation processes, which are critical in biotechnological applications ranging from wastewater treatment to bioremediation and the production of biochemicals [46,47].

Various primary models (Figs. 2 to 10) were utilized to fit the growth rate, and most of them show visually acceptable fitting. The best model based on statistical analysis was modified Gompertz model with the highest value for the adjusted coefficient of determination and the lowest values for RMSE and AICc and accuracy and bias factors were in optimal range (Table 2). Modelling results indicate phenol from 250 to 2250 mg/L as a sole carbon source is toxic, slowing bacterial growth at higher concentrations resulting in an increase in lag periods ranging from 5.8 to 9.4 hours (Fig. 11). The model was found to conform to normality tests and is adequate to be used to fit the experimental data. The normality tests carried out show that the model passes the normality tests with p > 0.05 for all normality tests carried out [48]. The experimental data obtained indicates that phenol is toxic and slows down the growth rate at higher concentrations. The modified Gompertz model fitting the growth of the bacterium at various concentrations of phenol (Fig. 12) and its resultant parameters are listed in Table 3.



Fig. 2. Modelling the growth of *Bacillus* sp. strain Neni-10 on 1000 mg/L phenol using the Huang model.



Fig. 3. Modelling the growth of *Bacillus* sp. strain Neni-10 on 1000 mg/L phenol using the Baranyi-Roberts model.



Fig. 4. Modelling the growth of *Bacillus* sp. strain Neni-10 on 1000 mg/L phenol using the modified Gompertz model.



Fig. 5. Modelling the growth of *Bacillus* sp. strain Neni-10 on 1000 mg/L phenol using the Buchanan-3-phase model.



Fig. 6. Modelling the growth of Bacillus sp. strain Neni-10 on 1000 mg/L phenol using the modified Richards model.



Fig. 7. Modelling the growth of Bacillus sp. strain Neni-10 on 1000 mg/L phenol using the modified Schnute model.



Fig. 8. Modelling the growth of Bacillus sp. strain Neni-10 on 1000 mg/L phenol using the modified Logistics model.



Fig. 9. Modelling the growth of Bacillus sp. strain Neni-10 on 1000 mg/L phenol using the von Bertalanffy model.



Fig. 10. Modelling the growth of Bacillus sp. strain Neni-10 on 1000 mg/L phenol using the MMF model.

Table 2. Statistical analysis of the growth models.

Model	р	RMSE	adR^2	AF	BF	AICc
Huang	4	0.1439	0.985	1.0319	1.0054	44.93
Baranyi-Roberts	4	0.2002	0.970	1.0780	0.9977	49.55
nodified Gompertz	3	0.0654	0.997	1.0400	1.0007	-8.10
Buchanan-3-phase	3	0.1594	0.983	1.0254	1.0009	4.38
nodified Richards	4	0.3126	0.932	1.1986	0.9293	55.79
nodified Schnute	4	0.1593	0.982	1.1986	0.9293	46.35
nodified Logistics	3	0.3108	0.943	1.3159	0.8471	13.72
on Bertalanffy	3	0.3725	0.923	1.8386	0.6036	16.26
MMF	4	0.142	0.985	1.052	0.999	44.75

Note: parameter р

RMSE Root Mean Square Error R² Coefficient of Determination adR²

Adjusted Coefficient of Determination Corrected Akaike Information Criterion

AICC BF

AF

Bias Factor Accuracy Factor Not available n.a.



Fig. 12. Lag period of *Bacillus* sp. strain Neni-10 at various phenol concentrations as modelled using the modified Gompertz model.



Fig. 12. Fitting the growth rate of *Bacillus* sp. strain Neni-10 at various phenol concentrations using the modified Gompertz model.

Table 3. Fitted parameters on the specific growth rate of *Bacillus* sp. strain Neni-10 using the modified Gompertz model.

		1000	1250	1500	1750	2000	2250
	500 mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Lag (h)	5.865	6.189	7.84	8.515	8.888	9.051	9.441
Y _{max}	3.785	3.733	2.964	1.602	1.225	0.954	0.807
$\mu_{max}\left(h^{\text{-}1}\right)$	0.26	0.311	0.18	0.068	0.032	0.022	0.012

In microbial kinetics, accurately modeling bacterial growth and the inhibitory effects of substrates is essential for optimizing bioprocesses, ensuring product safety, and understanding microbial ecology. Primary models like the modified Gompertz, modified Logistic, modified Richards, Baranyi-Roberts, modified Schnute, von Bertalanffy, Morgan-Mercer-Flodin (MMF), and Huang models are pivotal in this endeavor. These models describe bacterial growth under non-inhibitory conditions, estimating vital parameters such as specific growth rate (μ_m), lag phase duration, and maximum population density.

Understanding these parameters is crucial for advancing to more complex secondary modeling, which incorporates inhibitory effects using models like Haldane, Andrews, Yano, and Aiba. These primary models are instrumental in determining key growth parameters, fundamental in microbiology and biochemical engineering, defining the replication speed of bacteria under specific conditions. By providing detailed insights into bacterial growth dynamics, these models enable researchers to predict how bacteria will respond to various environmental changes and nutrient availability, which is vital for applications such as wastewater treatment, bioremediation, and the production of biofuels and other bioproducts [49–53].

The modified Gompertz model is a classical growth model, akin to the Verhulst model, and is widely used in microbial growth modeling [54,55]. Named after Benjamin Gompertz, the Gompertz function was described in the early 19th century and is based on an exponential relationship between specific growth rate and population density. The growth pattern described by this model is initially exponential; it then decelerates as saturation begins, and finally, growth ceases at maturity. Gibson et al. [56] were pioneers in applying the Gompertz equation to fit microbial growth curves. They successfully utilized the equation to describe the exponential and stationary phases of sigmoidal microbial growth curves. However, the original Gompertz model was insufficient to account for the lag phase, a critical initial period where growth is not observable as bacteria adapt to new conditions. To address this limitation, Gibson and colleagues modified the Gompertz model to incorporate the lag phase [56].

This modification allowed the model to more accurately represent the complete bacterial growth cycle, including the lag, exponential, and stationary phases. The modified Gompertz model has since been extensively used to model various microbial growth curves, solidifying its dominance in the mathematical modeling of bacterial growth and product formation [55,57,58]. The modified Gompertz model has been extensively used to model the growth of bacteria and the production of various bacterial secondary products. These include methane, biohydrogen, biofuel, lactic acid and bacteriocin, among others [59–63]. Additionally, it has been employed in modeling Mo-blue production in various bacteria. The model's ability to accurately represent the lag, exponential, and stationary phases of microbial growth makes it a versatile tool for studying diverse bioprocesses.

For instance, in biohydrogen production, the modified Gompertz model helps in understanding the hydrogen yield and production rate, essential for optimizing the bioprocess. In methane production, it aids in predicting the methane yield from anaerobic digestion processes. Similarly, for lactic acid and biofuel production, the model provides insights into the fermentation kinetics, crucial for scaling up the production processes. In bacteriocin production, the model helps in optimizing the conditions for maximum yield of these antimicrobial peptides. The model's widespread application in these areas underscores its utility in both research and industrial contexts, providing a reliable framework for optimizing microbial growth and product formation processes [50,64,65].

When a three-parameter model suffices to describe data, experts recommend it over a four-parameter model due to its simplicity and ease of use. The solution is more stable as the parameters are less correlated. Additionally, three-parameter models provide more degrees of freedom, which is crucial when dealing with growth or generation curves with a small number of measured points. It is also essential that all three parameters can be biologically interpreted, ensuring the model's relevance and accuracy in biological contexts. Parameters derived from model fitting exercises are biologically meaningful coefficients used in secondary modeling efforts. These mechanistic models are crucial in basic research, enhancing our understanding of the physical, chemical, and biological processes behind observed growth profiles. Mechanistic models are inherently more powerful when conditions are constant, as they provide insights into the underlying processes driving observed patterns. This foundation closely mimics biological systems, making these models particularly effective and reliable for extrapolating beyond initially observed conditions. The simplicity and ease of use of three-parameter models facilitate quicker understanding and implementation, while their stability, due to less correlated parameters, ensures consistent results across different datasets [66]. With fewer parameters to estimate, these models provide more degrees of freedom, allowing for more accurate and reliable parameter estimation, especially in small datasets.

Biological interpretation of model parameters ensures relevance and accuracy in representing biological phenomena, enhancing utility in scientific research. Mechanistic models help researchers dissect complex biological processes, leading to discoveries that inform practical applications in biotechnology, medicine, and environmental science. They are effective for predictive modeling because they closely mimic biological systems, making them valuable tools for scientific research and practical applications [67].

CONCLUSION

In conclusion, the study of bacterial growth on phenol exposes a unique phase where the specific growth rate initiates at zero and gradually accelerates to a maximum value, indicating a distinct lag period. This phase, a preparatory adjustment period for bacterial cells, is critical for understanding how bacteria adapt to new environmental conditions. Primary modeling of microbial growth, essential for determining key growth parameters like the maximum specific growth rate (µm), provides foundational insights for secondary modeling. Such insights are crucial for biotechnological applications, from wastewater treatment to bioremediation and biochemical production. The experimental data, supported by various primary models, indicates that phenol is toxic and inhibits bacterial growth at higher concentrations. Among the models tested, the modified Gompertz model demonstrated the best fit based on statistical analysis, normality tests, and key parameters such as the adjusted coefficient of determination, RMSE, AICc, accuracy, and bias factors. The model parameters especially the value of μ_m will be utilized in future publication to model the inhibitroy effect of phenol on the growth rate of this bacterium. Thus, the study provides valuable insights into microbial growth kinetics, crucial for optimizing biotechnological processes involving bacterial adaptation and growth under stress conditions.

REFERENCES

- Dahalan FA, Yunus I, Johari WLW, Shukor MY, Halmi MIE, Shamaan NA, et al. Growth kinetics of a diesel-degrading bacterial strain from petroleum-contaminated soil. J Environ Biol. 2014;35(2):399–406.
- Gami AA, Shukor MY, Khalil KA, Dahalan FA, Khalid A, Ahmad SA. Phenol and its toxicity. J Environ Microbiol Toxicol. 2014;2(1):11–23.
- Hansch C, McKarns SC, Smith CJ, Doolittle DJ. Comparative QSAR evidence for a free-radical mechanism of phenol-induced toxicity. Chem Biol Interact. 2000;127(1):61–72.
- Aditiawati P, Akhmaloka, Astuti DI, Sugilubin, Pikoli MR. Biodesulfurization of subbituminous coal by mixed culture bacteria isolated from coal mine soil of South Sumatera. Biotechnology. 2013;12(1):46–53.
- Yahuza S, Dan-Iya BI, Sabo IA. Modelling the Growth of Enterobacter sp. on Polyethylene. J Biochem Microbiol Biotechnol. 2020 Jul 31;8(1):42–6.
- 6. Rusnam, Yakasai HM, Rahman MF, Gusmanizar N, Shukor MY. Mathematical Modeling of Molybdenum-Blue Production from

Bacillus sp. strain Neni-. Bioremediation Sci Technol Res. 2021 Jul 31;9(1):7–12.

- Yakasai MH, Manogaran M. Kinetic Modelling of Molybdenumblue Production by *Bacillus* sp. strain Neni-10. J Environ Microbiol Toxicol. 2020 Jul 31;8(1):5–10.
- López S, Prieto M, Dijkstra J, Dhanoa MS, France J. Statistical evaluation of mathematical models for microbial growth. Int J Food Microbiol. 2004;96(3):289–300.
- McKellar RC, Knight K. A combined discrete-continuous model describing the lag phase of Listeria monocytogenes. Int J Food Microbiol. 2000;54(3):171–80.
- Kim HW, Lee SA, Yoon Y, Paik HD, Ham JS, Han SH, et al. Development of kinetic models describing kinetic behavior of *Bacillus cereus* and *Staphylococcus aureus* in milk. Korean J Food Sci Anim Resour. 2013;33(2):155–61.
- Li MY, Sun XM, Zhao GM, Huang XQ, Zhang JW, Tian W, et al. Comparison of Mathematical Models of Lactic Acid Bacteria Growth in Vacuum-Packaged Raw Beef Stored at Different Temperatures. J Food Sci. 2013;78(4):M600–4.
- Zwietering MH, Jongenburger I, Rombouts FM, Van't Riet K. Modeling of the bacterial growth curve. Appl Environ Microbiol. 1990;56(6):1875–81.
- Buchanan RL, Whiting RC, Damert WC. When is simple good enough: A comparison of the Gompertz, Baranyi, and three-phase linear models for fitting bacterial growth curves. Food Microbiol. 1997;14(4):313–26.
- Mansur R, Gusmanizar N, Roslan MAH, Ahmad SA, Shukor MY. Isolation and characterisation of a molybdenum-reducing and Metanil yellow dye-decolourising *Bacillus* sp. strain Neni-10 in soils from West Sumatera, Indonesia. Trop Life Sci Res. 2017 Jan;28(1):69–90.
- Rusnam, Gusmanizar N, Rahman MF, Yasid NA. Characterization of a Molybdenum-reducing and Phenol-degrading Pseudomonas sp. strain Neni-4 from soils in West Sumatera, Indonesia. Bull Environ Sci Sustain Manag E-ISSN 2716-5353. 2022 Jul 31;6(1):1–8.
- Shukor MY, Gusmanizar N, Azmi NA, Hamid M, Ramli J, Shamaan NA, et al. Isolation and characterization of an acrylamidedegrading *Bacillus cereus*. J Environmental Biol. 2009;30(1):57–64.
- 17. Marquardt DW. An algorithm for least-squares estimation of nonlinear parameters. SIAM J Appl Math. 1963;11(2):431–41.
- Motulsky HJ, Ransnas LA. Fitting curves to data using nonlinear regression: a practical and nonmathematical review. FASEB J Off Publ Fed Am Soc Exp Biol. 1987 Nov;1(5):365–74.
- Halmi, MIE, Shukor MS, Johari W.L.W WLW, Shukor MY. Mathematical Modeling of the Growth Kinetics of *Bacillus* sp. on Tannery Effluent Containing Chromate. J Environ Bioremediation Toxicol. 2014;2(1):6–10.
- 20. Akaike H. Factor analysis and AIC. Psychometrika. 1987;52(3):317–32.
- 21. Ross T, McMeekin TA. Predictive microbiology. Int J Food Microbiol. 1994;23(3–4):241–64.
- Aravindhan R, Naveen N, Anand G, Rao JR, Nair BU. Kinetics of biodegradation of phenol and a polyphenolic compound by a mixed culture containing Pseudomonas Aeruginosa and Bacillus Subtilis. Appl Ecol Environ Res. 2014;12(3):615–25.
- Folsom BR, Chapman PJ, Pritchard PH. Phenol and trichloroethylene degradation by Pseudomonas cepacia G4: Kinetics and interactions between substrates. Appl Environ Microbiol. 1990;56(5):1279–85.
- Hasan SA, Jabeen S. Degradation kinetics and pathway of phenol by Pseudomonas and Bacillus species. Biotechnol Biotechnol Equip. 2015;29(1):45–53.
- Tomasi I, Artaud I, Bertheau Y, Mansuy D. Metabolism of polychlorinated phenols by Pseudomonas cepacia AC1100: Determination of the first two steps and specific inhibitory effect of methimazole. J Bacteriol. 1995;177(2):307–11.
- Magharbeh MK, Khleifat KM, Al-Kafaween MA, Saraireh R, Alqaraleh M, Qaralleh H, et al. Biodegradation of phenol by Bacillus simplex: Characterization and kinetics study. Appl Environ Biotechnol. 2021;6(2):1–12.
- Ke Q, Zhang Y, Wu X, Su X, Wang Y, Lin H, et al. Sustainable biodegradation of phenol by immobilized Bacillus sp. SAS19 with porous carbonaceous gels as carriers. J Environ Manage. 2018;222:185–9.

- Chris Felshia S, Aswin Karthick N, Thilagam R, Chandralekha A, Raghavarao KSMS, Gnanamani A. Efficacy of free and encapsulated Bacillus lichenformis strain SL10 on degradation of phenol: A comparative study of degradation kinetics. J Environ Manage. 2017;197:373–83.
- Karthika S, Reshma MJ, Wilson PA, Das RA, Sarma US, Harikrishnan K, et al. Characterization and Evaluation of Phenol Degrading Bacillus Spp. for Enhancing the Softness of Coir Fiber. J Nat Fibers. 2016;13(3):253–60.
- Hasan SA, Jabeen S. Degradation kinetics and pathway of phenol by *Pseudomonas* and *Bacillus* species. Biotechnol Biotechnol Equip. 2015 Jan 2;29(1):45–53.
- Halmi MIE, Shukor MS, Johari WLW, Shukor MY. Mathematical modelling of the degradation kinetics of *Bacillus cereus* grown on phenol. J Environ Bioremediation Toxicol. 2014;2(1):1–5.
- Banerjee A, Ghoshal AK. Phenol degradation by Bacillus cereus: Pathway and kinetic modeling. Bioresour Technol. 2010;101(14):5501–7.
- Bai J, Wen JP, Li HM, Jiang Y. Kinetic modeling of growth and biodegradation of phenol and m-cresol using Alcaligenes faecalis. Process Biochem. 2007;42(4):510–7.
- Kiliç NK. Enhancement of phenol biodegradation by Ochrobactrum sp. isolated from industrial wastewaters. Int Biodeterior Biodegrad. 2009;63(6):778–81.
- Ahmad SA, Syed MA, Arif NM, Shukor MYA, Shamaan NA. Isolation, identification and characterization of elevated phenol degrading Acinetobacter sp. strain AQ5NOL 1. Aust J Basic Appl Sci. 2011;5(8):1035–45.
- Yadzir ZHM, Shukor MY, Nazir MS, Abdullah MA. Characterization and identification of newly isolated Acinetobacter baumannii strain Serdang 1 for phenol removal. In 2012. p. 223–8.
- Patil AH, Mishra RM, Kundar RR, Pendse AS. Study of phenol degrading bacterium isolated from a petrochemical contaminated site. J Appl Biol Sci. 2023 May 31;17(2):306–19.
- Wen Y, Li C, Song X, Yang Y. Biodegradation of phenol by rhodococcus sp. Strain SKC: Characterization and kinetics study. Molecules. 2020;25(16).
- Kumari S, Chetty D, Ramdhani N, Bux F. Phenol degrading ability of Rhodococcus pyrinidivorans and Pseudomonas aeruginosa isolated from activated sludge plants in South Africa. J Environ Sci Health - Part ToxicHazardous Subst Environ Eng. 2013;48(8):947– 53.
- Arif NM, Ahmad SA, Syed MA, Shukor MY. Isolation and characterization of a phenol-degrading *Rhodococcus* sp. strain AQ5NOL 2 KCTC 11961BP. J Basic Microbiol. 2013;53(1):9–19.
- Shumkova ES, Solyanikova IP, Plotnikova EG, Golovleva LA. Phenol degradation by *Rhodococcus opacus* strain 1G. Appl Biochem Microbiol. 2009;45(1):43–9.
- Nagamani A a, Lowry M b. Phenol biodegradation by Rhodococcus coprophilus isolated from semi arid soil samples of Pali, Rajasthan. Int J Appl Environ Sci. 2009;4(3):295–302.
- Čejková A, Masák J, Jirků V, Veselý M, Pátek M, Nešvera J. Potential of Rhodococcus erythropolis as a bioremediation organism. World J Microbiol Biotechnol. 2005;21(3):317–21.
- Halmi MIE, Wasoh H, Sukor S, Ahmad SA, Yusof MT, Shukor MY. Bioremoval of molybdenum from aqueous solution. Int J Agric Biol. 2014;16(4):848–50.
- 45. Baranyi J, Roberts TA. A dynamic approach to predicting bacterial growth in food. Int J Food Microbiol. 1994;23(3–4):277–94.
- Agarry SE, Audu TOK, Solomon BO. Substrate inhibition kinetics of phenol degradation by *Pseudomonas fluorescence* from steady state and wash-out data. Int J Environ Sci Technol. 2009;6(3):443– 50.
- Othman AR, Bakar NA, Halmi MIE, Johari WLW, Ahmad SA, Jirangon H, et al. Kinetics of molybdenum reduction to molybdenum blue by *Bacillus* sp. strain A.rzi. BioMed Res Int. 2013;2013:Article number 371058.
- Halmi MIE, Shukor MS, Masdor NA, Shamaan NA, Shukor MY. Testing the normality of residuals on regression model for the growth of *Paracoccus* sp. SKG on acetonitrile. J Environ Bioremediation Toxicol. 2015;3(1):15–7.
- Manogaran M, Othman AR, Shukor MY, Halmi MIE. Modelling the Effect of Heavy Metal on the Growth Rate of an SDS-degrading *Pseudomonas* sp. strain DRY15 from Antarctic soil. Bioremediation Sci Technol Res. 2019 Jul 31;7(1):41–5.

- Shukor MS, Shukor MY. Bioremoval of toxic molybdenum using dialysis tubing. Chem Eng Res Bull. 2015;18(1):6–11.
- Sevinç P, Gündüz U, Eroglu I, Yücel M. Kinetic analysis of photosynthetic growth, hydrogen production and dual substrate utilization by *Rhodobacter capsulatus*. Int J Hydrog Energy. 2012;37(21):16430–6.
- 52. McClure PJ, Cole MB, Davies KW. An example of the stages in the development of a predictive mathematical model for microbial growth: the effects of NaCl, pH and temperature on the growth of Aeromonas hydrophila. Int J Food Microbiol. 1994;23(3–4):359– 75.
- Dalgaard P. Modelling of microbial activity and prediction of shelf life for packed fresh fish. Int J Food Microbiol. 1995;26(3):305–17.
- Gompertz B. On the nature of the function expressive of the law of human mortality, and on a new mode of determining the value of life contingencies. Philos Trans R Soc London. 1825;115:513–85.
- Zwietering MH, Jongenburger I, Rombouts FM, Van't Riet K. Modeling of the bacterial growth curve. Appl Environ Microbiol. 1990;56(6):1875–81.
- Gibson AM, Bratchell N, Roberts TA. The effect of sodium chloride and temperature on the rate and extent of growth of Clostridium botulinum type A in pasteurized pork slurry. J Appl Bacteriol. 1987;62(6):479–90.
- Johnsen AR, Binning PJ, Aamand J, Badawi N, Rosenbom AE. The Gompertz function can coherently describe microbial mineralization of growth-sustaining pesticides. Environ Sci Technol. 2013;47(15):8508–14.
- López S, Prieto M, Dijkstra J, Dhanoa MS, France J. Statistical evaluation of mathematical models for microbial growth. Int J Food Microbiol. 2004;96(3):289–300.
- Espeche MC, Tomás MSJ, Wiese B, Bru E, Nader-Macías MEF. Physicochemical factors differentially affect the biomass and bacteriocin production by bovine Enterococcus mundtii CRL1656. J Dairy Sci. 2014;97(2):789–97.
- Kargi F, Eren NS, Ozmihci S. Effect of initial bacteria concentration on hydrogen gas production from cheese whey powder solution by thermophilic dark fermentation. Biotechnol Prog. 2012;28(4):931–6.
- Karthic P, Joseph S, Arun N, Varghese LA, Santhiagu A. Biohydrogen production using anaerobic mixed bacteria: Process parameters optimization studies. J Renew Sustain Energy. 2013;5(6).
- Mathias SP, Rosenthal A, Gaspar A, Aragão GMF, Slongo-Marcusi A. Prediction of acid lactic-bacteria growth in Turkey ham processed by high hydrostatic pressure. Braz J Microbiol. 2013;44(1):23–8.
- Mohammadi M, Mohamed AR, Najafpour GD, Younesi H, Uzir MH. Kinetic studies on fermentative production of biofuel from synthesis gas using clostridium ljungdahlii. Sci World J. 2014;2014.
- 64. Mansur R, Gusmanizar N, Dahalan FA, Masdor NA, Ahmad SA, Shukor MS, et al. Isolation and characterization of a molybdenumreducing and amide-degrading *Burkholderia cepacia* strain neni-11 in soils from west Sumatera, Indonesia. IIOAB. 2016;7(1):28–40.
- Yakasai MH, Ibrahim KK, Yasid NA, Halmi MIE, Rahman MFA, Shukor MY. Mathematical modelling of molybdenum reduction to mo-blue by a cyanide-degrading bacterium. Bioremediation Sci Technol Res. 2016 Dec 31;4(2):1–5.
- Burnham KP, Anderson DR. Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach. Springer Science & Business Media; 2002. 528 p.
- 67. Bolker BM. Ecological Models and Data in R. Princeton, N.J: Princeton University Press; 2008. 408 p.