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Primary Mathematical Modeling of the Growth of Diesel by a Bacterium Isolated from a Hydrocarbon-contaminated Soil

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ABSTRACT

Mathematical modeling of microbial growth via nonlinear regression is essential for determining key parameters such as the maximum specific growth rate, which are foundational for secondary modeling. Models such as 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. A previously isolated diesel-degrading *Pseudomonas* sp. strain Neni-4 growth on diesel was modeled using the aforementioned primary models. Experimental data showed that diesel concentrations from 0.25 to 3.5% (v/v) are toxic, slowing bacterial growth and increasing lag periods from 3 to 15 hours. Among the primary models tested, the Baranyi-Roberts 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 reliability of the Baranyi-Roberts model underscores its suitability for modeling bacterial growth under toxic conditions, offering valuable insights for optimizing biotechnological processes involving bacterial adaptation and growth under stress conditions.

INTRODUCTION

Hydrocarbons, such as oil, grease, and diesel, rank high among scheduled industrial wastes, second only to heavy metals [1]. Diesel and hydrocarbon compounds are hazardous to humans and other organisms, irritating mucous membranes, skin, eyes, and respiratory tract. Diesel pollution has been a significant environmental issue due to its widespread use and potential for causing severe ecological damage. Several notable diesel pollution incidents highlight the global impact of this problem. Diesel pollution in Antarctica, primarily from research stations and tourist vessels, poses significant risks to the pristine environment. Diesel spills can have long-lasting effects due to the continent's extreme cold, which slows down the natural degradation of pollutants. Studies have shown that diesel pollution adversely affects penguin populations and other wildlife in the region [2]. The explosion of the Deepwater Horizon drilling rig in the Gulf of Mexico resulted in the release of approximately 4.9 million barrels of oil, causing extensive environmental damage to marine and coastal ecosystems.

In 2001, a significant environmental disaster occurred in the Straits of Malacca when the Indonesian tanker MV Endah Lestari capsized, weighing 533 tons and carrying 18 tons of diesel and 600 tons of phenol. This accident resulted in the contamination of the coastal waters of Indonesia and Malaysia. The spill had a devastating impact on the local marine environment, killing thousands of fish and other marine organisms, including those raised in 85 offshore cages used for aquaculture. The toxic nature of diesel and phenol exacerbated the environmental damage, posing serious threats to both marine life and coastal ecosystems [3]

This disaster underscored the risks associated with offshore drilling operations and led to widespread calls for stricter regulations and safety measures in the oil industry. One of the most devastating oil spills in history occurred when the Exxon Valdez tanker struck a reef in Prince William Sound, Alaska, releasing 10.8 million gallons of crude oil. This spill resulted in massive environmental damage, killing thousands of seabirds, otters, and other wildlife. The incident significantly changed U.S. oil spill prevention and response policies.

More recently, The Japanese bulk carrier MV Wakashio ran aground off the coast of Mauritius, spilling approximately 1,000 tonnes of oil into the Indian Ocean. The spill had severe impacts on the island's coral reefs, mangroves, and marine biodiversity, highlighting the vulnerability of small island nations to maritime pollution. A case of continuing hydrocarbon pollution is seen in the Niger Delta, where it has experienced chronic oil pollution for decades due to leaks and spills from pipelines and infrastructure operated by multinational oil companies. This has caused long-term environmental degradation, affecting local communities' livelihoods and health. Efforts to clean up the pollution and hold companies accountable continue to face significant challenges [4–8]. These incidents underscore the importance of stringent regulations and effective response mechanisms to mitigate the environmental impact of diesel spills. Continued research and international cooperation are essential to address the challenges posed by diesel pollution and protect vulnerable ecosystems worldwide.

Diesel, a pollutant generated in various industrial processes, is composed of complex hydrocarbons, primarily with an aromatic benzene ring structure. Due to artificial contamination, it accumulates in soil, rivers, and groundwater, causing toxicity to both animals and plants and posing significant environmental concerns. Diesel's persistence in the environment is due to its hydrophobic nature and resistance to natural degradation processes. Various physicochemical methods are employed to remove diesel from wastewater, including chemical oxidation, which involves the use of strong oxidizing agents to break down diesel into less harmful substances. However, it can be expensive and may produce secondary pollutants. Another method is solvent extraction. This technique uses solvents to dissolve and extract diesel from contaminated water. While effective, it requires the safe disposal of the used solvents, which can be environmentally hazardous. Another method is adsorption by activated carbon.

Activated carbon is highly effective in adsorbing diesel from wastewater due to its large surface area and porosity. However, the regeneration and disposal of spent carbon can be challenging. Biological treatment, specifically using diesel-utilizing microorganisms, offers a more economical and efficient alternative. These microorganisms metabolize diesel, breaking it down into less harmful compounds through natural biochemical processes. Biological treatment avoids the risk of secondary contamination and is sustainable, leveraging the innate capabilities of bacteria and fungi to detoxify diesel pollutants [9–14]. Primary models effectively capture the nature of growth curves covering the lag, log (exponential), and stationary phases. This detailed comprehension is vital for predicting how bacteria respond to changes in their environment and nutrient availability. It is crucial to establish growth under controlled noninhibitory conditions before studying the effects of inhibitors. This sets a baseline for comparison in modeling efforts.

Once primary models outline growth under certain conditions, secondary models can forecast how inhibitors impact growth patterns. These secondary models consider factors like substrate inhibition, which's key for optimizing bioprocesses. When combined primary and secondary models create a framework that improves our ability to foresee and manage behavior across diverse biotechnological settings. Primary models play a role in kinetics by providing essential parameters and insights into bacterial growth under controlled circumstances.

Parameters such as growth rate (μ_m) lag phase duration and peak population density derived from primary models are crucial for secondary modeling focused on substrate inhibition dynamics. This knowledge is vital for optimizing bioprocesses spanning areas such as wastewater treatment, bioremediation and fermentation processes. The synergy between secondary models establishes a foundation for comprehending and steering microbial growth within industrial contexts and environmental scenarios. They allow for forecasts and fine tuning of operations, guaranteeing biotechnological activities' optimal performance and success [15–23]. This research aims to develop models that predict the development of *Pseudomonas* sp. strain Neni-4 on diesel. The models used include modified Gompertz, modified Logistic, modified Richards, Baranyi-Roberts, and modified Schnute models. The objective is to determine the most suitable model for the growth curve to gain a comprehensive insight into bacterial growth in these settings and enhance the precision of forecasts for improving biotechnological procedures related to diesel degradation.

MATERIALS AND METHODS

Growth medium for the diesel-degrading bacterium

A previously isolated phenol-degrading bacterium [24] was shown to grow on 1% (v/v) diesel as a carbon source. The growth characterization is published elsewhere. 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 diesel at various concentrations as the only carbon source and (mg/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. 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 sample from the bacterial culture was serially diluted using sterile tap water for subsequent enumeration of colony-forming units per milliliter (CFU/mL) [25].

Nonlinear curve fitting of the bacterial growth data

In this investigation, we used CurveExpert Professional (Version 1.6) software to analyze bacterial growth on diesel. This program use the Marquardt method to minimize the sum of squares of the disparities between anticipated and measured values. The Marquardt algorithm is an iterative technique that modifies parameters to minimize the discrepancy between projected and actual data, guaranteeing an ideal alignment with the growth curve. Using this strategy, we wanted to determine the best accurate main model for characterizing bacterial growth under these conditions (**Table 1**).

Table 1. Mathematical modeling of diesel growth by *Pseudomonas* sp. strain Neni-4.

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−λ), IF λ ≤ X ≥ X_{MAX} Y = 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
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, $A = \mu/\alpha$

Statistical analysis

The study included extensive error function studies, including Root-mean-square error (RMSE), Ross's bias factor (BF), accuracy factor (AF), and adjusted coefficient of determination (adjR^2) [26]. The rootmean-square error or RMSE was calculated according to Eq. 1;

The RMSE was calculated as follows,

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

where

n number of experimental data
Pd_i predicted values by the model
Ob_i experimental data
p parameters number of the model

Generally, models with fewer parameters tend to have lesser RMSE values [27]. Determining R^2, also known as the coefficient of determination, because it does not take into account the number of parameters of models, an alternative approach is to use an adjusted form of R^2 that has been modified to account for the large number of model parameters (**Eqns. 2** and **3)** of which it is used to work out the quality of nonlinear models according to the formula below.

$$Adjusted\left(R^2\right) = 1 - \frac{RMS}{S_Y^2} \quad \textbf{(Eqn. 2)}$$

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

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 a method for model selection that focuses on minimizing AIC values to choose the best model. Although a lower AIC value is often preferred, in some cases, an AICc value of -10 is more advantageous 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 [28]. AICc is calculated using the following equation (**Eqn. 4)**;

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

Where
n number of data points
p parameter numbers of the model

Equations 5 and 6, referred to as Accuracy Factor (AF) and Bias Factor (BF), are metrics utilized to evaluate the adequacy of models frequently employed in forecasting bacterial development in food science [29]. The statistics determine a perfect connection between experimental and projected results. A fail-safe model has a Benefit 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.

$$\text{Bias factor} = 10^{\left(\sum_{i=1}^{n}\log\frac{(Pd_i / Ob_i)}{n}\right)} \quad \textbf{(Eqn. 5)}$$

$$\text{Accuracy factor} = 10^{\left(\sum_{i=1}^{n}\log\frac{|(Pd_i / Ob_i)|}{n}\right)} \quad \textbf{(Eqn. 6)}$$

RESULTS AND DISCUSSION

The growth of the bacterium on diesel

Diesel-degrading bacteria are ideal for diesel remediation due to their cost-effectiveness and efficiency in breaking down hydrocarbons. Biodegradation of diesel by microorganisms has been a subject of intense research worldwide, driven by the need for environmentally friendly solutions to oil pollution. These bacteria utilize diesel as a carbon and energy source, breaking it down into less harmful substances. Research focuses on identifying effective strains, optimizing conditions for biodegradation, and understanding the metabolic pathways

involved. This knowledge enhances bioremediation strategies, making them more effective for cleaning diesel-contaminated environments and mitigating ecological damage. Key factors influencing biodegradation include microbial community composition, environmental conditions, and nutrient availability. Continuous advancements in this field promise to improve the sustainability and efficiency of diesel bioremediation efforts globally.

Bacteria that could degrade diesel include *Pseudomonas* species [9,30–39], *Bacillus* spp. [37,38,40–44], *Acinetobacter* spp. [11,13,31,45–51] and *Rhodococcus* species [37,52–59]. Each of these degraders has its own unique properties, such as the ability to tolerate high concentrations of diesel, salt tolerant, heavy metals tolerant, and the ability to grow at either extreme pHs or temperatures. The existence of many bacteria with diesel-degrading ability makes bioremediation the ideal method for diesel degradation. To date, very few primary models have been utilized. The growth of *Pseudomonas* sp. strain Neni-4 in the form of bacterial biomass on various concentrations of diesel was first converted to a natural logarithm (**Fig. 1**) before modeling. As the concentrations of diesel were increased, toxicity to growth was exhibited by an increase in the lag phase from 3 to 15 hours as well as a decline in biomass (**Fig. 1**).

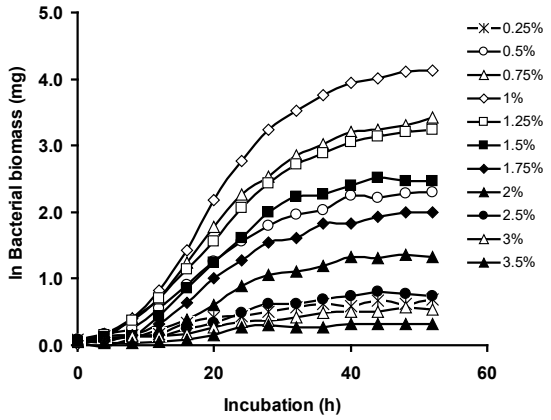

Fig. 1. Growth profile of *Pseudomonas* sp. strain Neni-4 on various concentrations of diesel.

It has been established that bacterial growth-linked processes, including growth on diesel, frequently display a unique phase in which the specific growth rate commences at a value of zero after which it accelerates to a maximal value (μ_{max}) in a certain time period, producing a lag time (λ) [60]. The sigmoidal shape commonly observed in bacterial growth curves is believed to feature a lag period. During this phase, bacterial cells adapt their growth mechanisms to new environmental conditions after a period of dormancy, particularly during storage. This preparatory phase is traditionally called the "lag period," when the cells adjust to new conditions before entering exponential growth. Baranyi and Roberts [61] described this phase as a transient period that links two autonomous growth systems. They posited that introducing the lag time or parameter in growth models serves primarily for convenience rather than a mechanistic explanation. It is hypothesized that individual bacterial cells exhibit varying growth rates within the initial inoculum.

These rates, if measurable, would likely display a nonlinear distribution, a concept supported by multiple researchers, including Baranyi and Roberts [61] and Buchanan et al. [23]. Primary modeling of microbial growth or product formation, such as in metal detoxification processes, is crucial as it helps in determining key growth parameters. 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 [62,63].

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 the Baranyi-Roberts 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 diesel from 0.5 to 3.5% (v/v) as a sole carbon source is toxic, slowing bacterial growth at higher concentrations and increasing lag periods ranging from 3 to 15 hours. The model was found to conform to normality tests and is adequate to be used to fit the experimental data. The normality tests showed that the model passed the normality tests with p >0.05 for all normality tests carried out [64]. The experimental data obtained indicates that diesel is toxic and slows down the growth rate at higher concentrations. The Baranyi-Roberts model fitting the growth of the bacterium at various concentrations of diesel (**Fig. 11**) and its resultant parameters are listed in **Table 3**.

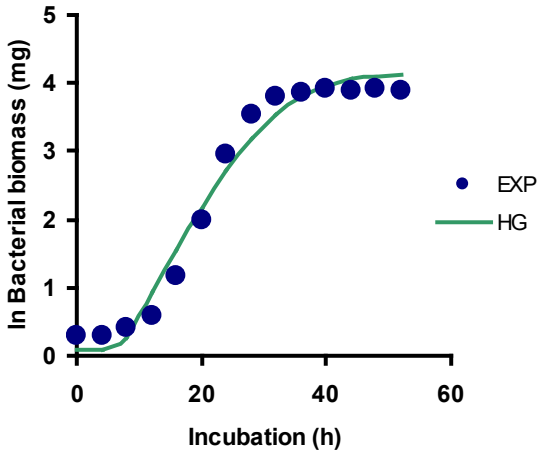

Fig. 2. Fitting the growth of *Pseudomonas* sp. strain Neni-4 on 1% (v/v) diesel using the Huang model.

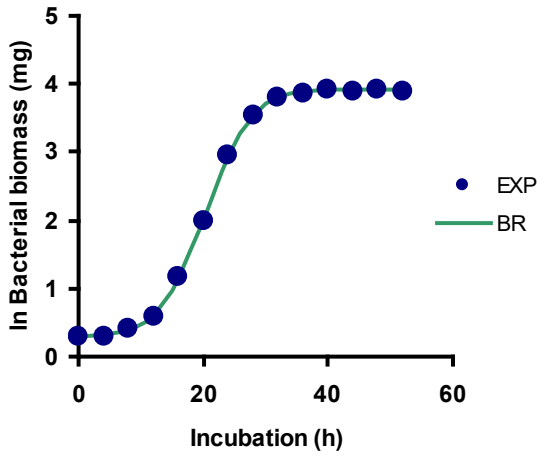

Fig. 3. Fitting the growth of *Pseudomonas* sp. strain Neni-4 on 1% (v/v) diesel using the Baranyi-Roberts model.

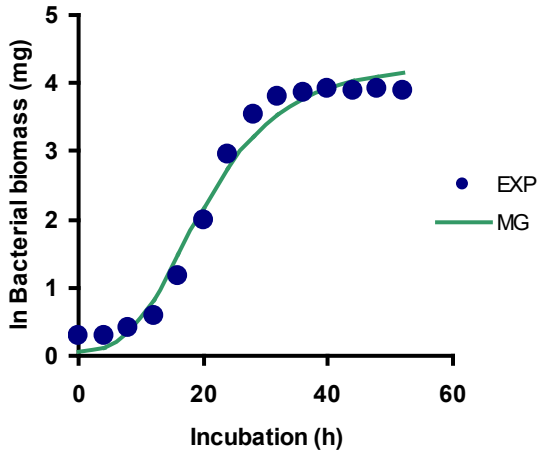

Fig. 4. Fitting the growth of *Pseudomonas* sp. strain Neni-4 on 1% (v/v) diesel using the modified Gompertz model.

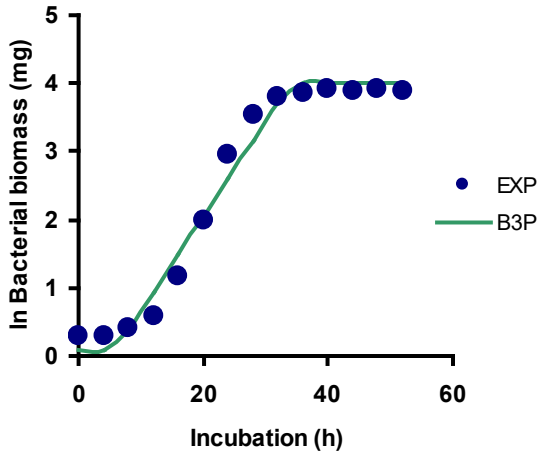

Fig. 5. Fitting the growth of *Pseudomonas* sp. strain Neni-4 on 1% (v/v) diesel using the Buchanan-3-phase model.

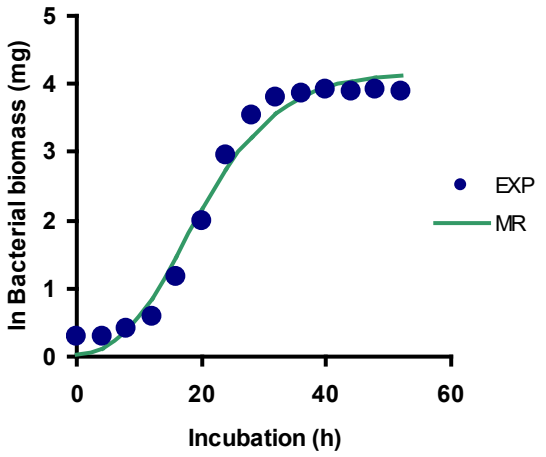

Fig. 6. Fitting the growth of *Pseudomonas* sp. strain Neni-4 on 1% (v/v) diesel using the modified Richards model.

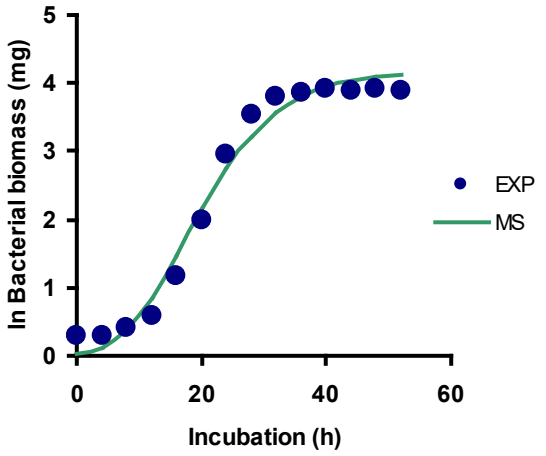

Fig. 7. Fitting the growth of *Pseudomonas* sp. strain Neni-4 on 1% (v/v) diesel using the modified Schnute model.

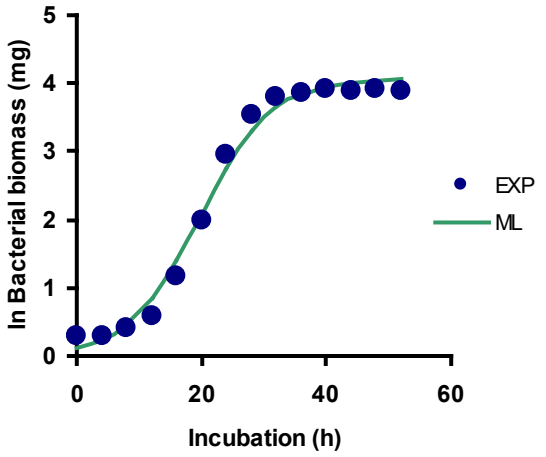

Fig. 8. Fitting the growth of *Pseudomonas* sp. strain Neni-4 on 1% (v/v) diesel using the modified Logistics model.

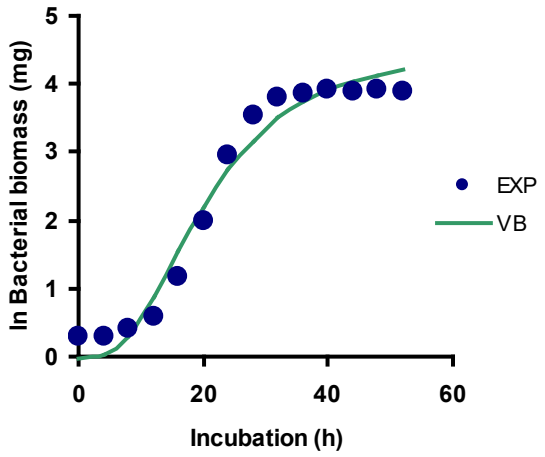

Fig. 9. Fitting the growth of *Pseudomonas* sp. strain Neni-4 on 1% (v/v) diesel using the von Bertalanffy model.

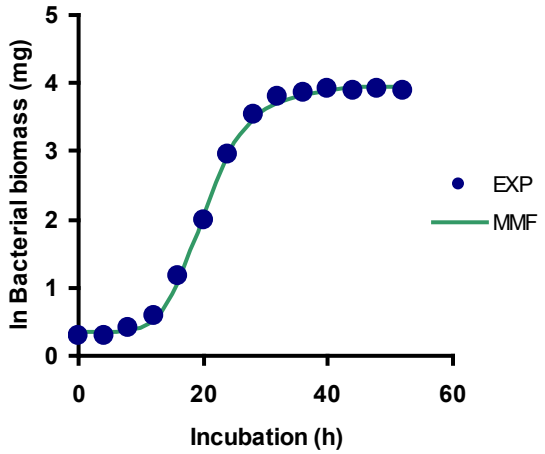

Fig. 10. Fitting the growth of *Pseudomonas* sp. strain Neni-4 on 1% (v/v) diesel using the MMF model.

Table 2. Error function analysis of the growth models utilized.

Model	*p*	RMSE	adR^2	AF	BF	AICc
Huang	4	0.2794	0.966	1.3556	0.8480	-14.91
Baranyi-Roberts	4	0.0200	1.000	1.0181	0.9991	-88.72
modified Gompertz	3	0.2342	0.976	1.3089	0.8520	-25.58
Buchanan-3-phase	3	0.2418	0.975	1.3089	0.8664	-24.69
modified Richards	4	0.2422	0.974	1.3498	0.8310	-18.91
modified Schnute	4	0.2407	0.975	1.3498	0.8310	-19.09
modified Logistics	3	0.1749	0.986	1.1565	0.9712	-33.76
von Bertalanffy	3	0.2898	0.965	1.3631	0.8400	-19.61
MMF	4	0.067	0.984	1.045	0.997	-55.07

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
n.a. Not available

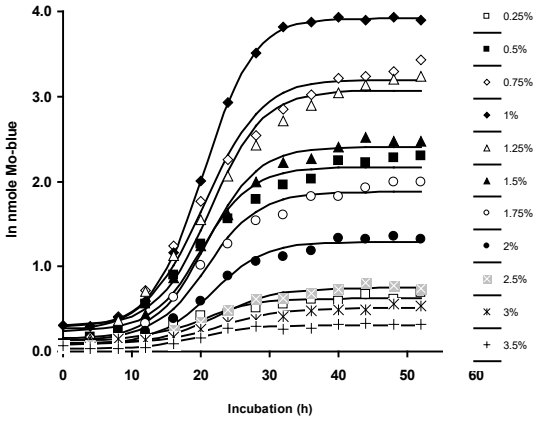

Fig. 11. Curve fitting of the growth rate of *Pseudomonas* sp. strain Neni-4 at various diesel concentrations using the Baranyi-Roberts model.

Table 3. Resultant parameters of the specific growth rate of *Pseudomonas* sp. strain Neni-4 using the Baranyi-Roberts model.

	0.25%	0.5%	0.75%	1%	1.25%	1.5%	1.75%	2%	2.5%	3%	3.5%
Y_0	0.136	0.244	0.266	0.303	0.311	0.159	0.14	0.081	0.096	0.088	0.032
Y_{max}	0.627	2.164	3.195	3.916	3.063	2.399	1.876	1.292	0.748	0.512	0.312
μ_{max} (h^{-1})	0.259	0.295	0.276	0.334	0.297	0.294	0.28	0.261	0.178	0.168	0.159

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 such as the modified Gompertz, modified Logistic, modified Richards, Baranyi-Roberts, modified Schnute, von Bertalanffy, Morgan-Mercer-Flodin (MMF), and Huang models play a crucial role in this endeavor. These models describe bacterial growth under noninhibitory conditions, estimating vital parameters such as the specific growth rate (μ_m), lag phase duration, and maximum population density.

Understanding these parameters is crucial for advancing to more complex secondary modeling, incorporating inhibitory effects using models like Haldane, Andrews, Yano, and Aiba. Primary models are instrumental in determining key growth parameters, fundamental in microbiology and biochemical engineering, as they define the replication speed of bacteria under specific conditions. This detailed understanding helps predict how bacteria respond to various environmental changes and nutrient availability, which is vital for wastewater treatment and bioremediation applications. Despite the importance of primary models, there is a notable gap in studies focusing on diesel biodegradation by microorganisms.

Few studies have utilized primary models to determine the specific growth rate needed for secondary models, such as Haldane, Teissier, and Aiba. This gap suggests an opportunity for future research to leverage primary models more extensively in the context of diesel biodegradation, improving the accuracy and efficiency of bioprocess optimization. By establishing bacterial growth under controlled, noninhibitory conditions through primary models, researchers can create a robust baseline for comparative analysis in secondary modeling.

These secondary models are then used to predict how various inhibitors affect growth kinetics, offering valuable insights for biotechnological applications. Together, primary and secondary models form an integrated framework that enhances our ability to predict and manipulate microbial behavior in diverse industrial and environmental settings [61,65–71]. The Baranyi-Roberts model initially suggested that a first-order differential equation (Eqn. 7) explains how the cell population (x) changes over time [72];

$$\frac{dx}{dt} = \alpha(t)\mu(x)x \qquad \textbf{(Eqn. 7)}$$

The following relationship for the production or growth rate is assumed (Eqn. 8)

$$\mu = \mu_{\max}\left(1 - \frac{x}{x_{\max}}\right) \qquad \textbf{(Eqn. 8)}$$

The generic form of the model can be rewritten as

$$\mu(t) = \frac{1}{x(t)}\frac{dx}{dt} = \mu_{\max}\alpha(t)f(t) \qquad \textbf{(Eqn. 9)}$$

The α(t) function in the model posits that development in the lag phase is hindered by a bottleneck intracellular substance denoted as P(t). The inhibitory mechanism resembles the Michaelis-Menten kinetics. The quotient q0 denotes the inoculum's physiological condition. The substance P(t) and its Michaelis-Menten constant increase exponentially from an initial value q_0 at a constant and defined pace. The $\alpha(t)$ increases monotonously with the limits $0 \leq \alpha \leq 1$ and $\lim_{t\to\infty} \alpha(t)=1$ as follows (Eqn 10);

$$\alpha(t) = \frac{P(t)}{P(t)+K_p} = \frac{q(t)}{1+q(t)} = \frac{q_0}{q_0 + e^{-\mu_{\max}t}} \qquad \textbf{(Eqn. 10)}$$

The end-of-growth or end-of product formation inhibition is represented by the $f(t)$ function (Eqn. 11). It decreases monotonically with $f(0) = 1$ and $\lim_{t\to\infty} f(t) = 0$. The $f(t)$ function is described by a logistic inhibition function in most dynamics models as follows;

$$f(t) = 1 - \left(\frac{x}{x_{\max}}\right) \qquad \textbf{(Eqn. 11)}$$

The differential equation was solved satisfactorily under specific fixed circumstances, such as constant temperatures (isothermal conditions). The penalty of the solution is due to its six parameters (Eqn. 12) [73];

$$y = A + \mu_{\max}x + \frac{1}{\mu_{\max}}\ln\left(e^{-\nu x} + e^{-h_0} - e^{-\nu x - h_o}\right) - \frac{1}{m}\ln\left(1 + \frac{e^{m\mu_{\max}x + \frac{1}{\mu_{\max}}\ln\left(e^{-\nu x} + e^{-h_0} - e^{-\nu x - h_0}\right)} - 1}{e^{m(y_{\max} - A)}}\right)$$

(Eqn. 12)

Where;
A represents the initial cell concentration (or product concentration), y_{max} is the asymptomatic cell concentration (or product concentration) in ln (c.f.u./mL or other growth units) or ln product concentration, the curvature parameter is m, and this characterizes the transition from the exponential phase. The initial physiological state of the cells is represented by h_o, a dimensionless parameter and the curvature parameter to characterize the transition to the exponential phase is represented by v. The lag time $\lambda(h)$ equals h_o/μ_{max}. The maximum specific growth rate $(1/h)$ is represented as μ_{max} or μ_m. The curvature parameters are suggested as follows; $v = \mu_{max}$ or μ_m and $m=1$ decreasing the number of parameters by two and resulting in the model having only four parameters; μ_{max}; h_0; A and y_{max} (Eqn. 13). Baranyi and Roberts suggested that h_0 may be regarded as a suitability indicator of the microorganism population towards the true environment [73]. When the experimental method is standardized, this suitability indicator may well be more or less constant which can be equal to the assumption that the lag time λ and the maximum specific growth rate μ_{max} are inversely proportional.

$$y = A + \mu_{\max}x + \frac{1}{\mu_{\max}}\ln\left(e^{-\mu_{\max}x} + e^{-h_0} - e^{-\mu_{\max}x - h_o}\right) - \ln\left(1 + \frac{e^{\mu_{\max}x + \frac{1}{\mu_{\max}}\ln\left(e^{-\mu_{\max}x} + e^{-h_0} - e^{-\mu_{\max}x - h_0}\right)} - 1}{e^{(y_{\max} - A)}}\right) \qquad \textbf{(Eqn. 13)}$$

The Baranyi-Roberts model is more mechanistic than the modified Gompertz model, as its parameters can be assigned biological meaning. Although the Baranyi-Roberts model requires fitting four parameters, it offers a more detailed and mechanistic understanding of bacterial growth. This model was chosen to fit the growth profile of the bacterium due to its mechanistic properties, which provide a more accurate representation of the biological processes involved. To increase the statistical significance of a four-parameter mechanistic model over a three-parameter non-mechanistic model, it is recommended to increase the number of data sets obtained. This approach enhances the robustness and reliability of the model, allowing for more precise parameter estimation and better insights into microbial growth dynamics [74].

The Baranyi and Roberts model has been successfully used to model microbial growth curves including *Brochothrix thermosphacta*, *Escherichia coli* O157:H7, *Bacillus* spp., *Listeria monocytogenes*, *Clostridium* spp., *Salmonella* Typhimurium, *Staphylococcus* spp. and *Yersinia enterocolitica* [73,75–78]. The Baranyi-Roberts model is preferred for several reasons. Firstly, it exhibits excellent fitting capabilities, providing a highly accurate representation of bacterial growth data. Secondly, the model is appropriate for dynamic environmental situations, making it versatile and robust under varying conditions. Thirdly, the majority of the model's parameters have biological meaning, allowing for a more mechanistic understanding of the growth processes. This biological relevance enhances the model's interpretability and usefulness in predicting and optimizing microbial growth in biotechnological applications [78,79]. The Baranyi-Roberts model has represented algal growth well in many studies [80,81].

Experts advise using a three-parameter model instead of a four-parameter model when it adequately describes the data, citing its simplicity and user-friendliness. The solution is more stable due to reduced parameter correlation. Furthermore, three-parameter models offer increased degrees of freedom, which are essential for analyzing growth or generation curves with a limited amount of data points. All three parameters must have a biological interpretation to ensure the model's relevance and accuracy in biological situations. Parameters obtained from model fitting exercises are significant coefficients that are

utilized in subsequent modeling endeavors. Mechanistic models play a vital role in fundamental research by deepening our comprehension of the underlying physical, chemical, and biological mechanisms that drive observable development patterns. Mechanistic models are more effective when conditions remain constant because they offer a deeper understanding of the fundamental mechanisms that influence observable patterns. This foundation closely imitates biological processes, making these models successful and dependable for predicting outcomes beyond the first observed conditions [82].

CONCLUSION

In conclusion, the study of bacterial growth on diesel reveals a unique phase where the specific growth rate starts 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 is essential for determining key growth parameters like the maximum specific growth rate, providing 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 diesel is toxic and inhibits bacterial growth at higher concentrations. Among the models tested, the Baranyi-Roberts 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's conformity to normality tests and adequacy in fitting experimental data highlight its reliability in modeling bacterial growth under toxic conditions. Thus, the study provides valuable insights into microbial growth kinetics, which is crucial for optimizing biotechnological processes involving bacterial adaptation and growth under stress conditions.

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