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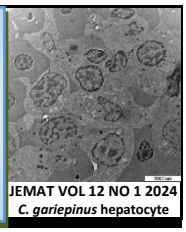

Primary Mathematical Modeling of the Growth of SDS by a bacterium Isolated From a Paddy Field

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ABSTRACT

The maximum specific growth rate and other critical parameters can be obtained from the mathematical modelling of microbial growth on toxic cubstances using nonlinear regression of several models. These models form the basis for secondary modelling in predictive microbiology. NumerousiImportant biotechnological applications like bioremediation and wastewater treatment rely on models that explain how substrates affect bacterial growth and biotransformation processes. These models include the modified Gompertz, modified Logistic, modified Richards, Buchanan-3-phase, Baranyi-Roberts, modified Schnute, von Bertalanffy, Morgan-Mercer-Flodin (MMF), and Huang. A previously isolated SDS-degrading *Pseudomonas* sp. strain Maninjau1 was studied for its growth on SDS using these primary models. Experimental data showed that SDS concentrations from 0.25 to 2.2 g/L (250 to 2200 mg/L) were toxic, slowing bacterial growth. As SDS concentrations increased, toxicity was evident, marked by an increase in the lag phase from 5.9 to 6.9 hours and a decline in biomass. The highest growth rate was observed at 1000 mg/L SDS. The bet model based on error function discriminatory analysis is MMF, which outperformed other models that were tested. The reasons for this include a favorable accuracy (AF) and bias factor (BF), a low root-mean-square (RMSE) and AICc value, and a high adjusted coefficient of determination. The validity of the MMF model for simulating bacterial development in toxic environments is supported by its high reliability. Insights into how bacteria adapt and grow under stressful circumstances can greatly improve biotechnological processes.

INTRODUCTION

Detergents are widely recognized for their harmful impact on marine ecosystems [1–3]. Previous research have shown that anionic surfactants can endanger the health of several aquatic organisms at concentrations between 0.0025 to 300 mg/L [4]. The aquatic species' reproductive cycles and behavioral behaviors were impacted [5]. Another study revealed that the digestive gland of oysters is vulnerable to SDS exposure, leading to disturbances in the oyster's nutritional and metabolic functions, ultimately reducing its chances of survival [6]. Increased discharge of anionic surfactants into water bodies would result in elevated pollution levels, causing harm to a greater variety of invertebrate and crustacean species due to their toxic nature.

The concentration of detergents in household wastewater varies from 3 to 21 mg/L, whereas in some industrial effluents, it may be as high as 10,000 mg/L. The elevated levels of surfactants in the washing facility's effluent provide a significant challenge for treatment. Sodium dodecyl sulfate (SDS) is the most often used anionic surfactants and builders in laundry detergent manufacture. The concentration of detergents in laundry effluent varied from 17 mg/L to 1024 mg/L, as reported [7].

Microbes are recognized for their capacity to break down organic compounds such as SDS. Their role in bioremediation is crucial for the cost-effective elimination of xenobiotic contaminants [8]. *Pseudomonas* sp. strain C12B was one of the first bacteria examined for its ability to biodegrade anionic

surfactants under aerobic conditions [9], followed by several SDS-degrading bacteria [10–25] and in more recent studies [26–31]. Studies on cold-adapted microorganisms capable of degrading SDS are uncommon and were initially documented by Margesin and Schinner [15,32] and later by other workers [29,33,34].

Primary models accurately represent the characteristics of growth curves, including the lag, log (exponential), and stationary phases. Thorough understanding is crucial for anticipating how bacteria react to alterations in their surroundings and nutrition supply. It is essential to first ensure development in a controlled environment without inhibitors before investigating the impact of inhibitors. This establishes a standard for comparison in modeling endeavors. Primary models describe growth under certain settings, whereas secondary models predict how inhibitors affect growth patterns. These secondary models take into account issues such as substrate inhibition, which is crucial for improving bioprocesses. Combining primary and secondary models enhances our capacity to predict and control behavior in various biotechnology environments.

Primary models are crucial in kinetics since they offer vital characteristics and understanding of bacterial development in controlled conditions. Key parameters including growth rate (μ_m), lag phase length, and peak population density obtained from primary models are essential for secondary modeling that concentrates on substrate inhibition dynamics. This understanding is crucial for improving bioprocesses in fields like wastewater treatment, bioremediation, and fermentation. The interaction between secondary models forms a basis for understanding and controlling microbial development in industrial and environmental settings. They enable predictions and precise adjustments of operations to ensure the best performance and success of biotechnological activities [35–43]. This research intends to create predictive models for the growth of a previously isolated SDS-degrading bacterium using models such as 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. The goal is to identify the optimal 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 SDS degradation.

MATERIALS AND METHODS

Growth medium for the SDS-degrading bacterium

A previously isolated SDS-degrading bacterium near the Maninjau Lake West Sumatra, Indonesia [26] was utilized in this study. 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 a basal salts (BS) enrichment medium (g/L) contained the followings: KH_2PO_4, (1.36), Na_2HPO_4, (1.39), KNO_3, (0.5), $MgSO_4$ (0.01), $CaCl_2$ (0.01) and $(NH_4)_2SO_4$ (7.7). The pH was set at 7.0. Filter-sterilized sodium dodecyl sulphate was added into the medium as a carbon source [26]. For longer than two weeks storage, a slant nutrient agar plates supplemented with sodium dodecyl sulfate were used to maintain the bacterium's pure culture, which was incubated at 30 °C for up to six days and then stored in the fridge for up to two months. Liquid nitrogen is used to store an 80% glycerol stock for extended maintenance periods. The colony count method, with appropriate dilutions in autoclaved tap water, was used to measure the bacterial growth (CFU/mL). Bacterial biomass was measured in milligrams of dry biomass in accordance with [44].

Nonlinear curve fitting of the bacterial growth data

CurveExpert Professional (Version 1.6) software was utilized to examine bacterial growth on SDS in this study. This software use the Marquardt technique to minimize the sum of squares of the differences between expected and observed values. The Marquardt algorithm is an iterative method that adjusts parameters to reduce the difference between planned and observed data, ensuring optimal alignment with the growth curve. We aimed to identify the most precise primary model for defining bacterial growth under these conditions by this approach (**Table 1**).

Table 1. Mathematical modeling of growth on SDS by *Pseudomonas* sp. strain Maninjau1.

Model	p	Equation
Modified Logistic	3	$y = \frac{A}{1 + exp\left[\frac{4\mu_m}{A}(\lambda - t) + 2\right]}$
Modified Gompertz	3	$y = Aexp\left\{-exp\left[\frac{\mu_m . e}{A}(\lambda - t) + 1\right]\right\}$
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, $A = m/a$

Statistical analysis

The study involved thorough analysis of error functions such as Root-mean-square error (RMSE), Ross's bias factor (BF), accuracy factor (AF), and adjusted coefficient of determination (adjR^2) [45]. 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 [46]. 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.

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

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

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 [47]. 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 [48]. 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 SDS

SDS-degrading bacteria are ideal for SDS remediation due to their cost-effectiveness and efficiency in breaking down hydrocarbons. Biodegradation of SDS by microorganisms has been a subject of intense research worldwide, driven by the need for environmentally friendly solutions to oil pollution. These bacteria utilize SDS 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 SDS-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 SDS bioremediation efforts globally. Bacteria that could degrade SDS include *Pseudomonas* species [26,30,49–53], *Enterobacter* sp. [31], *Klebsiella oxytoca* [54] and *Delftia acidovorans* [24].

The capacity to grow at extreme pH levels or temperatures, tolerance of heavy metals, salt tolerance, and tolerance of high concentrations of SDS are just a few of the distinctive characteristics shared by these degraders. Since there are many different kinds of bacteria that can break down SDS, bioremediation is the best option for this process. To date very few primary models have been utilized. The growth of *Pseudomonas* sp. strain Maninjau1 in the form of bacterial biomass on various concentrations of SDS were first converted to natural logarithm (**Fig. 1**) before modelling. As the concentrations of SDS was increased, toxicity to growth was apparent exhibited by an increase in the lag phase from 0 to 12 hours as well as a declined in biomass. Growth was optimal at 1000 mg/L SDS (**Fig. 1**).

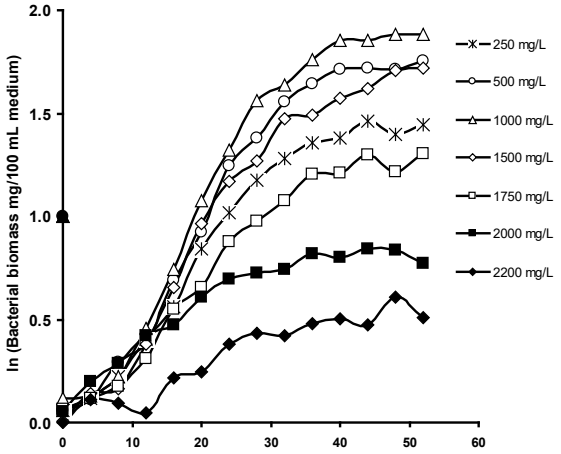

Fig. 1. Growth profile of *Pseudomonas* sp. strain Maninjau1 on various concentrations of SDS.

Bacterial growth processes, such as growth on SDS, often exhibit a distinct phase where the specific growth rate starts at zero and then increases to a maximum value (μ_{max}) in a certain time period, producing a lag time (λ) [55]. The sigmoidal form of bacterial growth curves is thought to include a lag time where bacterial cells adjust their growth processes to new environmental circumstances following a period of dormancy, especially during storage. During this preliminary phase, known as the "lag period," cells acclimate to new surroundings before starting exponential growth. Baranyi and Roberts [56] shows that this phase is characterized as a temporary time that connects two independent development systems. They suggested that incorporating a lag time or parameter into growth models is mostly for convenience rather than providing a mechanistic explanation.

It is theorized that individual bacterial cells in the first inoculum have different growth rates. If quantifiable, these rates would probably have a nonlinear distribution, a theory endorsed by several scholars such as Baranyi and Roberts [56] and Buchanan et al. [43]. Modeling microbial growth or product creation, especially in metal detoxification processes, is essential for identifying important growth factors. The resulting parameters, especially the maximal specific growth rate (μ_m), are crucial for future stages in secondary modeling. The characteristics are essential for correctly simulating microbial activity under different environmental conditions and pressures. Secondary models like Monod, Haldane, Aiba, and Teissier are commonly used in further studies to understand how substrates affect bacterial growth or the rates at which xenobiotics are transformed. These models play a crucial role in explaining how varying levels of substrates might impact microbial growth rates and biotransformation processes, which are essential in many biotechnological fields such as wastewater treatment, bioremediation, and biochemical synthesis [57,58].

The majority of the main models that were used to fit the growth rate (**Figs. 2–10**) provide visually acceptable results. With an adjusted coefficient of determination at its highest and RMSE and AICc values at their lowest, as well as accuracy and bias factors in the ideal range, the MMF model emerged as the top statistical model (**Table 2**). According to the results of the models, using SDS as the only carbon source from 0.25 to 2.2 g/L is harmful because it slows down the growth of bacteria at higher concentrations, leading to longer lag times ranging from 5.9 to 6.9 hours. The model passed the normalcy tests and is suitable for fitting the experimental data. All of the normalcy tests that were run on the model yielded p-values greater than 0.05, indicating that it passes the tests [59]. Experiment results show that SDS is toxic and exhibits a growth rate retardation effect at higher concentrations. **Table 3** contains the parameters that were determined by fitting the bacterium's growth to the MMF model at different concentrations of SDS (**Fig. 11**).

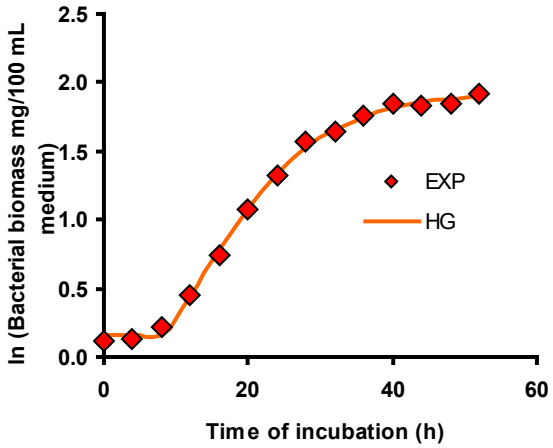

Fig. 2. Fitting the growth of *Pseudomonas* sp. strain Maninjau1 on 1% (v/v) SDS using the Huang model.

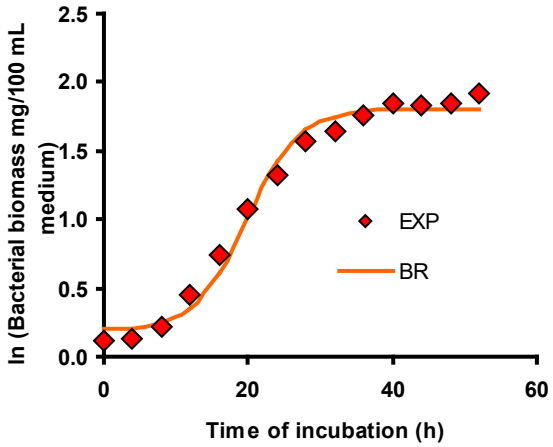

Fig. 3. Fitting the growth of *Pseudomonas* sp. strain Maninjau1 on 1% (v/v) SDS using the Baranyi-Roberts model.

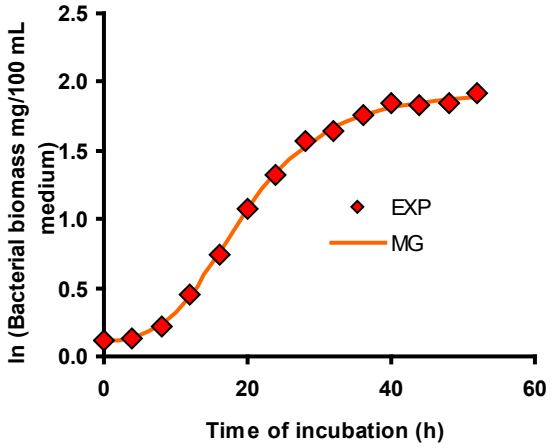

Fig. 4. Fitting the growth of *Pseudomonas* sp. strain Maninjau1 on 1% (v/v) SDS using the modified Gompertz model.

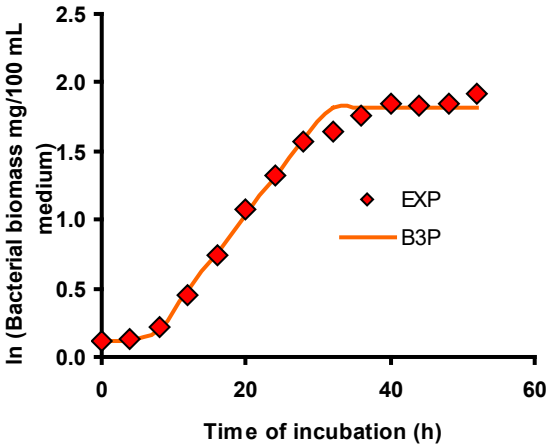

Fig. 5. Fitting the growth of *Pseudomonas* sp. strain Maninjau1 on 1% (v/v) SDS using the Buchanan-3-phase model.

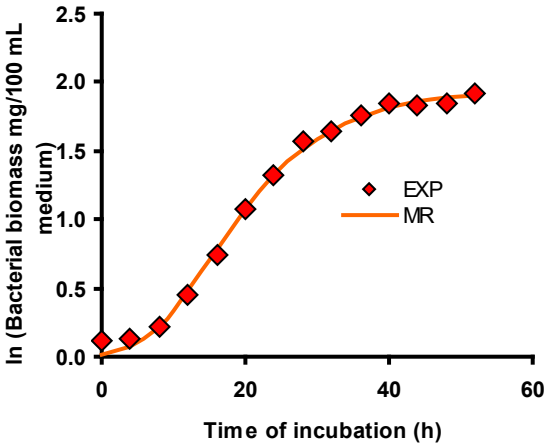

Fig. 6. Fitting the growth of *Pseudomonas* sp. strain Maninjau1 on 1% (v/v) SDS using the modified Richards model.

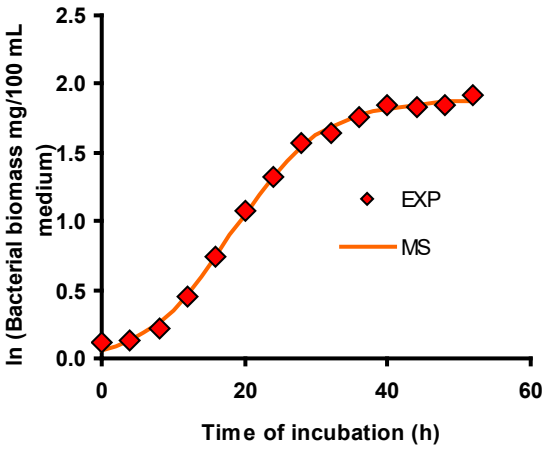

Fig. 7. Fitting the growth of *Pseudomonas* sp. strain Maninjau1 on 1% (v/v) SDS using the modified Schnute model.

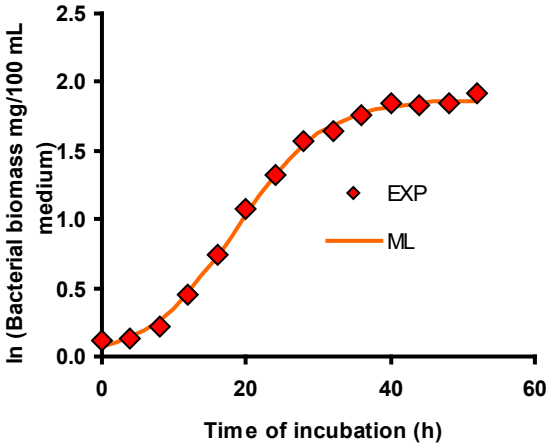

Fig. 8. Fitting the growth of *Pseudomonas* sp. strain Maninjau1 on 1% (v/v) SDS using the modified Logistics model.

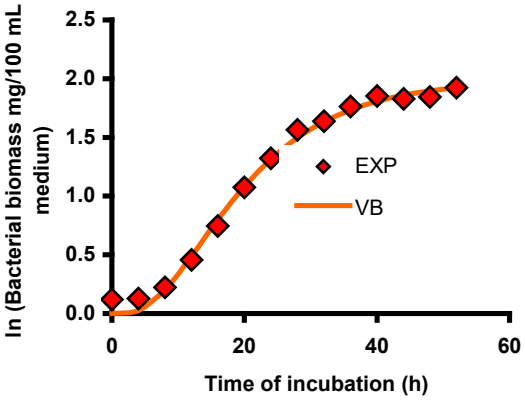

Fig. 9. Fitting the growth of *Pseudomonas* sp. strain Maninjau1 on 1% (v/v) SDS using the von Bertalanffy model.

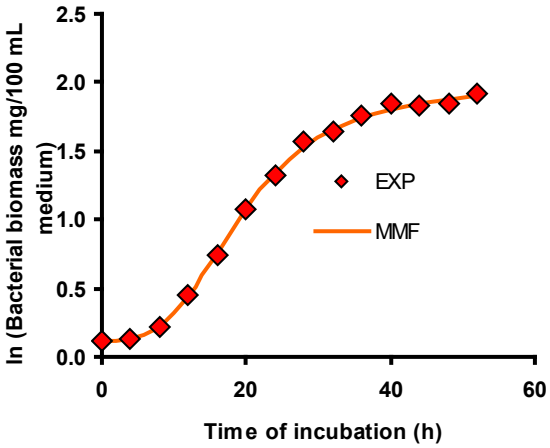

Fig. 10. Fitting the growth of *Pseudomonas* sp. strain Maninjau1 on 1% (v/v) SDS using the MMF model.

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

Model	*p*	RMSE	R^2	adR^2	AF	BF	AICc
Huang	4	0.0395	0.9976	0.996	1.0805	1.0113	-69.68
Baranyi-Roberts	4	0.1000	0.9842	0.977	1.1532	1.0505	-43.69
modified Gompertz	3	0.0920	0.9856	0.981	1.0370	1.0014	-51.74
Buchanan-3-phase	3	0.0686	0.9920	0.990	1.0370	0.9948	-59.95
modified Richards	4	0.0491	0.9964	0.995	1.2305	0.8152	-83.57
modified Schnute	4	0.0996	0.9847	0.978	1.0718	0.2643	-43.81
modified Logistics	3	0.0353	0.9979	0.997	1.0680	0.9940	-78.84
von Bertalanffy	3	0.0601	0.9942	0.992	1.5953	0.6441	-63.66
MMF	4	0.026	0.998	0.997	1.017	1.000	-80.89

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

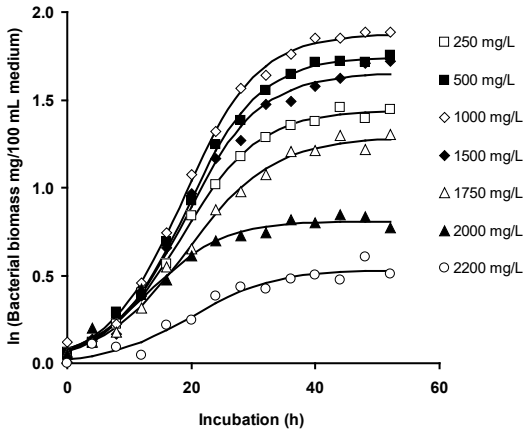

Fig. 11. Curve fitting of the growth rate of *Pseudomonas* sp. strain Maninjau1 at various SDS concentrations using the MMF model.

Table 3. Resultant parameters of the specific growth rate of *Pseudomonas* sp. strain Maninjau1 using the MMF model.

	250 mg/L	500 mg/L	1000 mg/L	1500 mg/L	1750 mg/L	2000 mg/L	2200 mg/L
Y_{max}	1.44	1.75	1.903	1.65	1.29	0.81	0.53
μ_{max} (h^{-1})	0.06	0.07	0.08	0.07	0.05	0.03	0.02
Lag (h)	5.93	7.28	6.75	6.85	5.61	6.40	6.91

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 are crucial in this endeavor. These models describe bacterial growth under non-inhibitory conditions, estimating vital parameters such as the specific growth rate, lag phase duration, and maximum population density.

Understanding these parameters is vital for advancing to more complex secondary modeling, which incorporates inhibitory effects using models like Haldane, Monod, Yano, Andrews, 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 crucial for applications such as wastewater treatment and bioremediation.

Despite the importance of primary models, there is a notable gap in studies focusing on the biodegradation of sodium dodecyl sulfate (SDS) 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 represents a opportunity for future research to leverage primary models more extensively in the context of SDS biodegradation, improving the accuracy and efficiency of bioprocess optimization. By establishing bacterial growth under controlled, non-inhibitory conditions through primary models, researchers can create a robust baseline for comparative analysis in secondary modeling. These secondary models can then be 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 [28,56,60–65].

The MMF model was first created to explain diverse nutrient-response connections in complex organisms [66]. So far, the model has been useful in various modeling exercises with animals like rabbits, sheep, horses, and microorganisms.[67–73], exopolysaccharide production by *Klebsiella oxytoca* [74], yeast [75]a yield of oil palm [76], ethanol [77], human deaths due to COVID-19 [78–83] and even in finance [84]. Parameters derived from model fitting exercises are important coefficients used in future modeling efforts. Mechanistic models are essential in basic research since they enhance our understanding of the fundamental physical, chemical, and biological systems responsible for observed patterns of development. Mechanistic models are most useful under constant settings as they provide a profound insight into the basic mechanics that impact observed patterns. This foundation closely mimics biological processes, rendering these models very effective and reliable in forecasting consequences beyond initial observed circumstances [85].

CONCLUSION

The investigation of bacterial growth on SDS demonstrates a characteristic lag time, characterized by the specific growth rate starting at zero and progressively increasing to a maximum value. This preliminary adjustment phase is crucial for comprehending how bacteria acclimate to new environmental circumstances. Modeling microbial growth is crucial for finding important growth characteristics such as the maximum specific growth rate, which is fundamental for further modeling. These insights are essential for several biotechnological uses, including wastewater treatment, bioremediation, and biochemical synthesis. The experimental evidence, backed by many main models, suggests that SDS is hazardous and hinders bacterial growth at elevated doses. The MMF model showed the most accurate fit among the models examined, as determined by statistical analysis, normality tests, and important metrics like the adjusted coefficient of determination, RMSE, AICc, accuracy, and bias factors. The model's adherence to normality tests and its suitability in fitting experimental data emphasize its dependability in simulating bacterial development in harmful environments. The work offers unique insights into microbial growth kinetics, essential for enhancing biotechnological processes that include bacterial adaptability and development under stressful circumstances.

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