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Primary Modeling of Microbial Growth under Toxic Conditions with the Modified Schnute Model

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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. These models, including those by Monod, Haldane, Aiba, and Teissier, elucidate the impact of substrates on bacterial growth and biotransformation processes, vital for biotechnological applications like wastewater treatment and bioremediation. Experimental data showed that acrylamide from 250 to 1250 mg/L as a sole nitrogen source is toxic, slowing bacterial growth at higher concentrations resulting in an increase in lag periods ranging from 3 to 9 hours. Various primary models were tested, with the modified Schnute model providing the best fit based on statistical analysis, normality tests, and key parameters such as adjusted coefficient of determination near to unity, lowest values for RMSE and AICc values and good values of accuracy (AF) and bias factors (BF). The modified Schnute model's reliability 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

In microbial kinetics, accurately modeling bacterial growth and the inhibitory effects of substrates on this growth is crucial for optimizing bioprocesses, ensuring product safety, and enhancing our understanding of microbial ecology. Primary models like the modified Gompertz, modified Logistic, modified Richards, Baranyi-Roberts, modified Schnute, von Bertalanffy, Morgan-Mercer-Flodin (MMF) and the Huang models play a pivotal role in this endeavor. These models help describe the growth characteristics of bacteria under non-inhibitory conditions, enabling the estimation of vital parameters such as the specific growth rate, lag phase duration, and maximum population density. Understanding these parameters is essential for advancing to more complex secondary modeling exercises that incorporate the inhibitory effects of substrates on microbial growth using models such as Haldane, Andrews, Yano, and Aiba. Primary models are instrumental in determining key growth parameters. For instance, the specific growth rate obtained from these models is fundamental in microbiology and biochemical engineering because it defines the speed at which bacteria replicate under specific conditions [1–5].

Primary models capture the sigmoidal nature of bacterial growth curves, including the lag, log (exponential), and stationary phases. This detailed understanding aids in predicting how bacteria respond to environmental changes and nutrient availability. Before exploring how inhibitors affect bacterial growth, it is necessary to establish how bacteria grow under controlled, non-inhibitory conditions. This baseline is crucial for comparative analysis in secondary modeling. Once primary modeling has adequately described growth under non-stressful

conditions, secondary models can be employed to understand and predict how various inhibitors affect growth kinetics: These models are specifically designed to incorporate substrate inhibition. 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.

Together, primary and secondary models form an integrated framework that significantly enhances our ability to predict and manipulate microbial behavior in various biotechnological applications [6–14]. The main objective of this research is to model the growth of a bacterium on the toxic substance acrylamide using several primary models mentioned above and finding the best model that fit the growth curve.

MATERIALS AND METHODS

All chemical reagents were generated in large quantities and utilised in the analysis in their unpurified forms, and all of the materials used in this study were of analytical grade. In all cases, unless otherwise noted, experiments were carried out in triplicate.

Growth and maintenance of acrylamide-degrading bacterium

The growth and maintenance of acrylamide-degrading bacterium is as before [15]. An aliquot of 0.1 mL from a freshly cultured overnight suspension of the bacterium in nutrient broth was transferred to 45 mL of acrylamide enrichment medium contained within a 100 mL volumetric flask. 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.

The growth medium used was Minimal Salt Medium (MSM), which included acrylamide at various concentrations as the exclusive nitrogen source, supplemented with glucose at 10 g/L for carbon, $MgSO_4 \cdot 7H_2O$ at 0.5 g/L, KH_2PO_4 at 6.8 g/L, and additional trace elements [4]. The pH of this medium was adjusted to the desired level. For sterilization purposes, PTFE syringe filters (0.45 micron) were employed, and acrylamide served as the sole nitrogen source. 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) [16].

Fitting of the bacterial growth data

We utilized CurveExpert Professional (Version 1.6) software in this study, which minimizes the sums of squares of the differences between predicted and measured values. The program utilizes a Marquardt algorithm (**Table 1**).

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) [17]. The rootmean-square error or RMSE was calculated according to Eq. 1;

Table 1. Mathematical modeling of the growh of acrylamide by Pseudomonas sp. strain DrY135.

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	$y = N_0 + \mu_m t + \frac{1}{\mu_m} ln(e^{-\mu_m t} + e^{-h_0} - e^{-\mu_m t - h_0}) - ln\left[1 + \frac{e^{\mu_m t + \frac{1}{\mu_m} ln\left(e^{-\mu_m t} + e^{-h_0} - e^{-\mu_m t - h_0}\right)}}{e^{(A - N_0)}}\right]$
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, $A = \mu/\alpha$

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

As general rule, those model that has smaller number of parameter corresponds in smaller RMSE value [18]. 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.

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

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

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 fundamentally an information-theoretic approach to model selection, focusing on minimizing the AIC values to select the optimal model. However, a lower AIC value might not always be the most suitable choice; for example, an AICc value of -10 is generally more favorable than a value of -1. The AIC integrates a penalty for increasing complexity, which reflects the addition of parameters, indicating a decrease in model simplicity. This penalization helps to prevent the selection of overly complicated models for fitting experimental data. Particularly when dealing with a small number of parameters, researchers might adopt a refined version known as the corrected AIC, or AICc, which facilitates more precise comparisons between models [19]. 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 [20]. 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

It has been established that bacterial growth-linked processes including growth on acrylamide 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) [21]. The sigmoidal shape commonly observed in bacterial growth curves is believed to feature a lag period because during this phase, bacterial cells are adapting their growth mechanisms to new environmental conditions after a period of dormancy, particularly during storage. This preparatory phase is traditionally referred to as the "lag period," a time when the cells adjust to new conditions before entering exponential growth. Baranyi and Roberts [22] described this phase as a transient period that links two autonomous growth systems. They posited that the introduction of the lag time or parameter in growth models serves primarily for convenience rather than offering a mechanistic explanation.

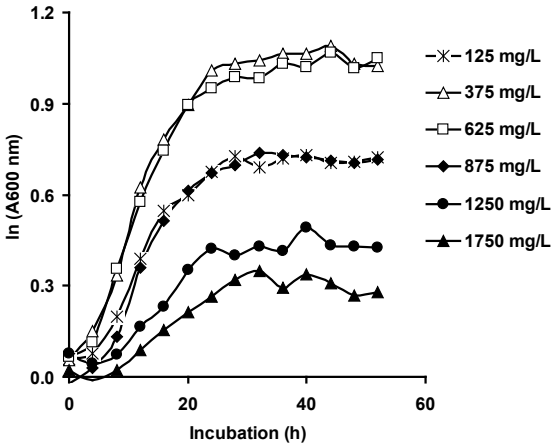

Fig. 1. The growth of *Pseudomonas* sp. strain DrY135 on various concentrations of acrylamide.

It is hypothesized that within the initial inoculum, individual bacterial cells exhibit varying growth rates. These rates, if measurable, would likely display a nonlinear distribution, a concept supported by multiple researchers including Baranyi and Roberts [22] and Buchanan et al. [14]. 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 [23,24].

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 Schnute 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**). 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 [25]. The experimental data obtained indicates that acrylamide is toxic and slows down the growth rate at higher concentrations. The modified Schnute model fitting the growth of the bacterium at various concentrations of acrylamide (**Fig. 11**) and its resultant parameters are listed in **Table 3**.

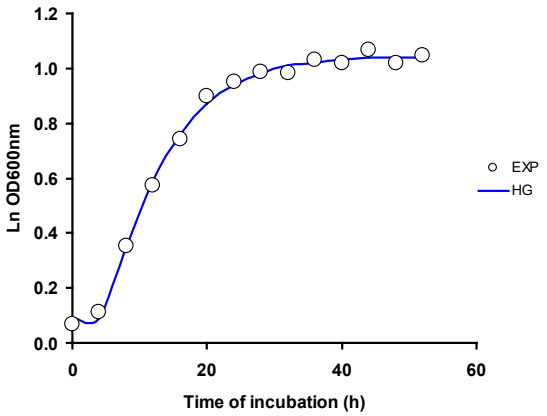

Fig. 2. Modelling the growth of Pseudomonas sp. strain DrY135 on 625 mg/L acrylamide using the Huang model.

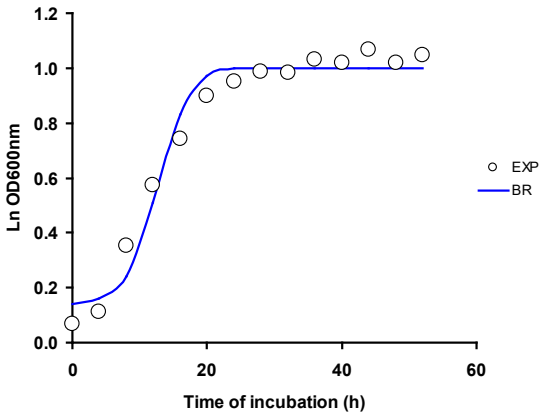

Fig. 3. Modelling the growth of Pseudomonas sp. strain DrY135 on 625 mg/L acrylamide using the Baranyi-Roberts model.

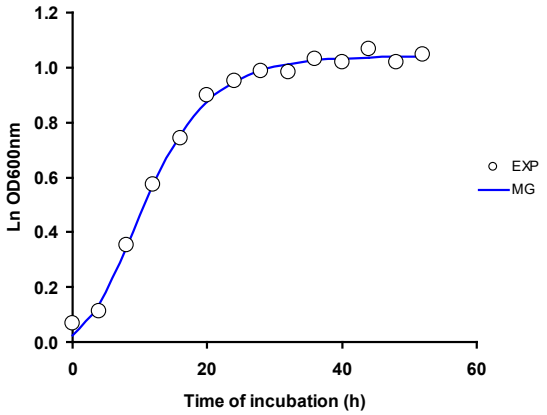

Fig. 4. Modelling the growth of Pseudomonas sp. strain DrY135 on 625 mg/L acrylamide using the modified Gompertz model.

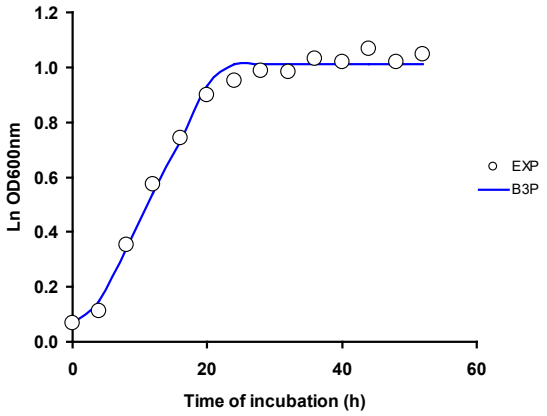

Fig. 5. Modelling the growth of Pseudomonas sp. strain DrY135 on 625 mg/L acrylamide using the Buchanan-3-phase model.

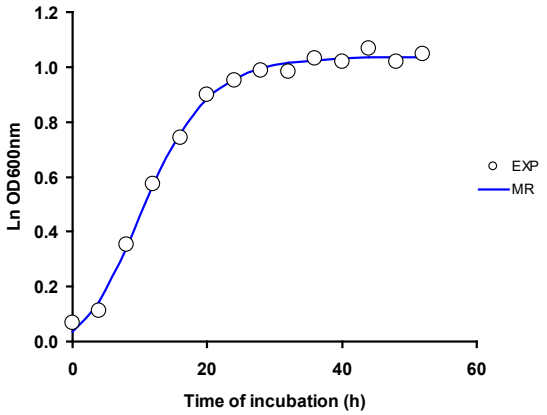

Fig. 6. Modelling the growth of Pseudomonas sp. strain DrY135 on 625 mg/L acrylamide using the modified Richards model.

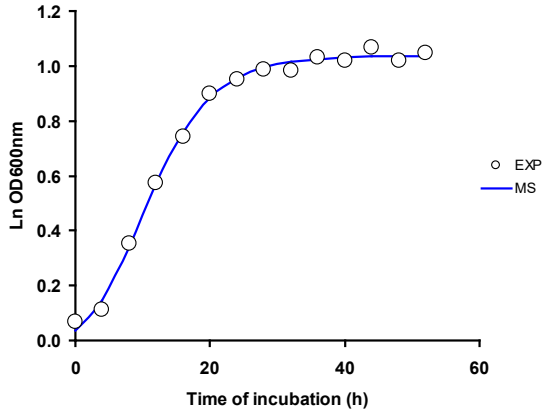

Fig. 7. Modelling the growth of Pseudomonas sp. strain DrY135 on 625 mg/L acrylamide using the modified Schnute model.

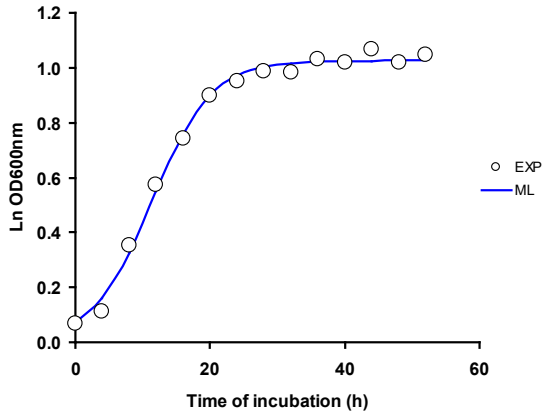

Fig. 8. Modelling the growth of Pseudomonas sp. strain DrY135 on 625 mg/L acrylamide using the modified Logistics model.

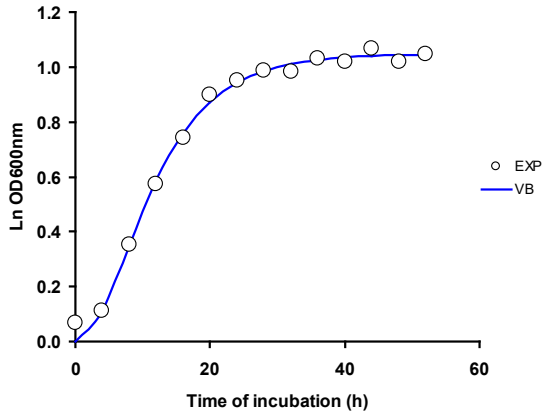

Fig. 9. Modelling the growth of Pseudomonas sp. strain DrY135 on 625 mg/L acrylamide using the von Bertalanffy model.

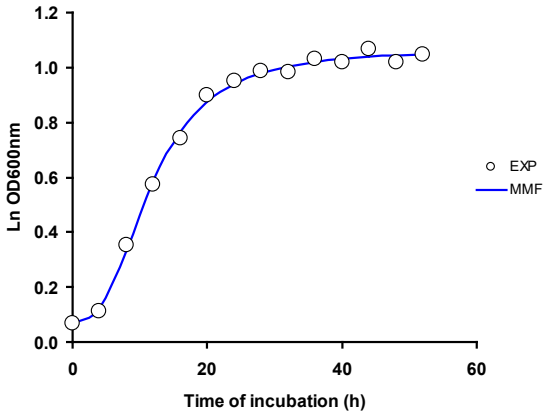

Fig. 10. Modelling the growth of Pseudomonas sp. strain DrY135 on 625 mg/L acrylamide using the MMF model.

Table 2. Statistical analysis of the growth models.

Model	*p*	RMSE	adR^2	AF	BF	AICc
Huang	4	0.022	0.996	1.027	0.999	-86.576
Baranyi-Roberts	4	0.070	0.955	1.077	1.008	-53.614
modified Gompertz	3	0.023	0.996	1.016	1.000	-91.034
Buchanan-3-phase	3	0.036	0.988	1.029	1.001	-77.965
modified Richards	4	0.023	0.995	1.018	0.999	-85.049
modified Schnute	4	0.023	0.997	1.018	1.000	-95.049
modified Logistics	3	0.028	0.993	1.023	0.997	-85.454
von Bertalanffy	3	0.027	0.994	1.036	0.997	-86.327
MMF	4	0.020	0.997	1.036	0.997	-89.06

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

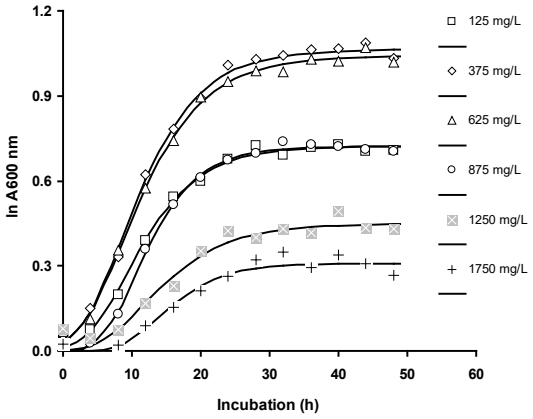

Fig. 11. Fitting the growth rate of *Pseudomonas* sp. strain DrY135 at various acrylamide concentrations using the modified Schnute model.

Table 3. Fitted parameters on the specific growth rate of *Pseudomonas* sp. strain DrY135 using the modified Schnute model.

	125 mg/L	375 mg/L	625 mg/L	875 mg/L	1250 mg/L	1750 mg/L
μ_{max}	0.052	0.103	0.059	0.057	0.032	0.02
β	-10.68	-5.90	-4.17	-10.11	-18.70	-3.86
α	0.361	0.303	0.222	0.379	0.419	0.086
Lag (h)	3.13	4.25	5.72	6.84	8.29	9.40

The Schnute model, originally developed for modeling fish growth, is distinguished by its ability to describe growth trajectories where the asymptotic size is not easily ascertainable, a scenario often encountered with fish as opposed to the typically clearer limiting growth exhibited by bacteria. This model shares similarities with the Richards model in that both can flexibly accommodate a range of growth patterns, including parabolic, concave, and sigmoidal shapes, depending on the specific coefficients derived from a given dataset. This versatility is due to the parametric flexibility inherent in these models, which allows them to adapt the growth curve's shape significantly based on the biological data observed. The ability to describe different types of growth curves makes the Schnute model particularly valuable across various biological disciplines [13,26–28].

In addition to its applications in aquaculture and fisheries science, the Schnute model has also been successfully applied in biotechnology, such as in modeling β-carotene production by *Dunaliella salina*. In this context, the model was compared to other well-known growth models like the modified Logistic, Gompertz, Richards, and Stannard models, demonstrating its utility in capturing the complex dynamics of bioprocesses beyond traditional applications. This broad applicability underscores the model's robustness and adaptability, making it a preferred choice for researchers dealing with non-standard growth phenomena where traditional models might fail to provide accurate or meaningful insights [29].

In a number of cases, the four-parameter Schnute model [30] was statistically better than the three-parameter Gompertz model in modelling the growth of *P. putida* and *E. agglomerans* [31]. The modified Schnute model was also a better model than other models such as von Bertalanffy, logistic, Gompertz and Schnute-Richards in modelling the growth of the Cortes geoduck *Panopea globosa* [32]. The Schnute model has also found application in modelling growth of forest species [33].

Parameters derived from the model fitting exercises are biologically meaningful coefficients that are subsequently utilized in secondary modeling efforts. These mechanistic models play a pivotal role in basic research, aiming to deepen our understanding of the physical, chemical, and biological processes that contribute to the observed growth profiles. Mechanistic models, when conditions are held constant, are inherently more powerful because they provide insights into the underlying processes that drive observed patterns. This makes them particularly effective and reliable when extrapolating beyond the conditions initially observed, as they are built on a foundation that closely mimics the biological systems they aim to represent [34].

CONCLUSION

In conclusion, the study of bacterial growth on acrylamide 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 acrylamide is toxic and inhibits bacterial growth at higher concentrations. Among the models tested, the modified Schnute 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 conformity of the modified Schnute model to the normality tests and its adequacy in fitting experimental data highlights its reliability in modeling bacterial growth under toxic conditions. Thus, the study provides valuable insights into microbial growth kinetics, crucial for optimizing biotechnological processes involving bacterial adaptation and growth under stress conditions.

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