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Kinetic Models of Microbial Growth Inhibition of *Pseudomonas* sp. on Acrylamide

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ABSTRACT

In this study, various secondary growth models, including Luong, Yano, Teissier-Edward, Aiba, Haldane, Monod, Han, and Levenspiel, were employed to model the inhibitory effect of high acrylamide concentrations on the growth rate of *Pseudomonas* sp. strain DrY135. Following thorough statistical analyzes, the ten bacterial growth models ranged from very poor fits, as observed with the Luong, Monod, and Webb models, to exceptionally good fits for the other models. The Han-Levenspiel model was superior, demonstrating minimal RMSE, BIC, HQC, and modified adj.R^2 values, except for the MPSD and AICc statistics. Moreover, the model's Accuracy Factor (AF) and Bias Factor (BF) values were close to unity, indicating a good fit between predicted and observed data. Experimental research indicates that acrylamide is detrimental and impedes growth at elevated concentrations. The Han-Levenspiel constants, including the maximal degradation rate (μmax), half-saturation constant (*Ks*), maximal substrate concentration tolerated (*Sm*), and curve-fitting parameters (*m* and *n*), were determined to be 16.704 h^{-1}, 3943.26 mg/L, 125.58 mg/L, 3.1469, and 0.9835, respectively. However, these values were accompanied by very large confidence intervals, likely due to the limited dataset. Similarly, the fitted parameters of other models also exhibited large 95% confidence intervals, likely for the same reason. Future remedies include incorporating additional data points to improve fitting accuracy. These enhanced constants can serve as significant inputs for future modeling projects. Furthermore, integrating substrate inhibition kinetics into risk assessment models can enhance the precision of hazard evaluation for toxic substrates at contaminated sites. This knowledge is vital for informed decision-making in environmental management.

INTRODUCTION

In microbial kinetics, precise modeling of bacterial growth and the inhibitory effects of substrates is essential for optimising bioprocesses, ensuring product safety, and advancing our understanding of microbial ecology. Primary models, such as the modified Gompertz, Logistic, Richards, Baranyi-Roberts, Schnute, von Bertalanffy, Morgan-Mercer-Flodin (MMF), and Huang models, play a critical role in this process. These models describe bacterial development in non-inhibitory environments, facilitating the estimation of critical parameters such as specific growth rate, lag phase duration, and maximum population density. Understanding these factors is crucial for advancing to more complex secondary modeling tasks that account for substrate inhibition using models such as Haldane, Andrews, Yano, and Aiba. Specific growth rates derived from these primary models are particularly important in microbiology and biochemical engineering, as they determine bacterial replication rates under defined conditions [1–5].

Fundamental models encapsulate the sigmoidal characteristics of bacterial growth curves, encompassing the lag, logarithmic (exponential), and stationary phases. This comprehensive understanding facilitates the prediction of bacterial responses to environmental fluctuations and nutritional accessibility. Prior to examining the impact of inhibitors on bacterial proliferation, it is essential to delineate the growth of bacteria under regulated, non-inhibitory settings. This baseline is essential for comparison analysis in secondary modeling. Once primary modeling has sufficiently characterized growth under optimal conditions, secondary models can be employed to analyse and predict the impact of various inhibitors on growth

kinetics. These models are explicitly engineered to integrate substrate inhibition. Primary models are essential in microbial kinetics as they are providing key characteristics and insights into bacterial development under controlled settings. These characteristics are essential for secondary models that emphasise substrate inhibition, which is crucial for thorough bioprocess optimization [6–8].

Substrate inhibition is a phenomena where elevated substrate concentrations adversely impact the growth or activity of microorganisms. Numerous mathematical models have been created to elucidate this effect, each providing distinct insights and applicability under varying experimental situations. Comprehending these models is crucial for precisely predicting microbial activity in industrial and environmental contexts. The Monod model, a prevalent framework, characterises microbial development in relation to substrate concentration. Although efficient at low substrate concentrations, Monod's model fails to address inhibition at elevated substrate levels. To mitigate this constraint, adaptations such as the Haldane model were created. The Haldane model incorporates an extra term to address substrate inhibition, accurately depicting situations when development diminishes beyond an ideal substrate concentration [9–11].

The Aiba model, akin to Haldane, emphasizes the integration of substrate inhibition in its formulation. It is especially appropriate for systems where the inhibitory mechanism entails competition for enzyme binding sites. The Yano and Teissier-Edward model expands on inhibition dynamics by incorporating the affinity of the enzyme-substrate complex, making it a powerful tool for understanding biological processes involving inhibition. The Luong model introduces the concept of a critical substrate concentration, where growth is optimal up to this threshold. Beyond this point, the growth rate sharply declines until total inhibition occurs. This model is particularly useful for microbiological systems with well-defined inhibition points, such as those involving hazardous substrates.

Han and Levenspiel's approach provides an alternative viewpoint, integrating a broader spectrum of substrate influences on growth. Their approach is adaptable and can characterise both the stimulating and inhibitory phases of substrate concentration. This makes it particularly advantageous for bioprocess optimization, as transitions between these stages often occur. The distinctive features of each model render them suitable for circumstances. The Teissier-Edward model is utilised in enzyme kinetics research, but the Luong model is prevalent in environmental engineering for the breakdown of dangerous compounds. Notwithstanding their advantages, these models are also accompanied by constraints. They frequently necessitate experimental data for parameter estimation and may inadequately characterize intricate, mixed-substrate systems [12–18].

Besides theoretical comprehension, these models facilitate the optimization of industrial operations. During bioremediation, comprehending substrate inhibition kinetics enables researchers to modify substrate concentrations to optimize microbial breakdown efficiency. Similarly, in fermentation processes, models such as Aiba and Haldane help mitigate inhibitory substrate concentrations that may result in production losses. Substrate inhibition kinetics models offer a framework for analysing and forecasting microbial growth under various situations.

The choice of a suitable model is contingent upon the system's particulars, including substrate properties and microbial traits. As these models advance, their utilisation remains crucial in disciplines such as bioprocess engineering and environmental sciences. The proper implementation of these models can result in substantial improvements in process optimization, however obstacles exist in their parameterisation and validation. The combination of primary and secondary models creates a cohesive framework that markedly improves our capacity to forecast and influence microbial activity in diverse biotechnological applications [19–27].

Acrylamide is a chemical molecule predominantly utilized in industrial applications, including water treatment, paper manufacturing, and plastic production. It is also generated during the preparation of starchy meals at elevated temperatures, presenting a possible risk to human health. Acrylamide is designated as a potential human carcinogen, and extended exposure may result in neurological consequences, reproductive complications, and developmental damage. Its environmental endurance, especially in aquatic and terrestrial ecosystems, amplifies its potential risks. The biodegradation of acrylamide has become a useful bioremediation approach due to its hazardous characteristics and environmental persistence. Numerous microorganisms, such as bacteria, fungi, and algae, have been recognized for their capacity to breakdown acrylamide via enzymatic mechanisms.

These microorganisms employ enzymes like acrylamidase and amidase to decompose acrylamide into less deleterious substances. The biodegradation process entails the transformation of acrylamide into non-toxic by-products, like acrylic acid or ammonia, rendering it a viable approach for the remediation of contaminated areas. This bioremediation technology provides an environmentally acceptable, economical, and sustainable alternative to conventional chemical techniques, which can be more costly and perilous.

Research persists in enhancing these microbial processes for extensive applications to reduce acrylamide contamination in industrial effluents and food processing waste [28–35]. The main objective of this research is to model the growth of a bacterium on the toxic substance acrylamide previously modelled using primary models [36] using several secondary 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 impurified forms, and all the materials used in this study were of analytical grade. In all cases, unless otherwise noted, experiments were carried out in triplicate.

Fitting of the bacterial growth data

The Schnute model parameters previously obtained [28] was utilized in this study. We utilized Curve Expert 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**).

Table 1. Substrate inhibition mathematical models.

Author	Degradation Rate	Author
Monod	$\frac{q_{max}S}{S+K_s}$	[29]
Haldane	$\frac{q_{max}S}{S+K_s+\left(\frac{S^2}{K_i}\right)}$	[30]
Teissier	$q_{max}\left(1-exp\left(-\frac{S}{K_i}\right)-exp\left(\frac{S}{K_s}\right)\right)$	[31]
Aiba	$q_{max}\frac{S}{K_s+S}exp\left(-\frac{S}{K_i}\right)$	[32]
Yano and Koga	$\frac{q_{max}S}{S+K_s+\left(\frac{S^2}{K_i}\right)\left(1+\frac{S}{K}\right)}$	[33]
Han and Levenspiel	$q_{max}\left(1-\left(\frac{S}{S_m}\right)\right)^n\left(\frac{S}{S+K_s\left(1-\left(\frac{S}{S_m}\right)\right)^m}\right)$	[34]
Luong	$q_{max}\frac{S}{S+K_s}\left(1-\left(\frac{S}{S_m}\right)\right)^n$	[35]
Moser	$\frac{q_{max}s^n}{K_s+s^n}$	[36]
Webb	$\frac{q_{max}S\left(1+\frac{S}{K}\right)}{S+K_s+\frac{S^2}{K_i}}$	[37]
Hinshelwood	$q_{max}\frac{S}{K_s+S}(1-K_pP)$	[38]

Note:
q_{max} maximal specific growth rate
K_s half saturation constant
K_i inhibition constant
S_m maximal concentration of substrate tolerated
K_p product inhibition constant
m, n, K curve parameters
S substrate concentration
p product concentration

Statistical analysis

The statistically significant difference between the models was evaluated using various metrics, The following statistical functions were utilized to determine the best models.

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

The quality of the nonlinear models was determined by adjusting the R^2 value. S_y^2 is the total variance of the y-variable, while RMS stands for residual mean square. These two terms are used in the adjusted R^2 formula (Equations 2 and 3).

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

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

The Akaike Information Criterion (AIC) helps compare statistical models for experimental data. Akaike created this criterion. Instead, data sets with many parameters or few values should use the corrected AIC (AICc) [39]. The AICc was determined using the equation that is presented below (Equation 4).

$$AICc = 2p + n1n\left(\frac{RSS}{n}\right) + 2(p+1) + \frac{2(p+1)(p+2)}{n-p-2} \quad \text{(Eqn. 4)}$$

The Bayesian Information Criterion (BIC) (Equation 5) is another information theory-based statistical metric like the AICc. The lowest Bayesian information criterion (BIC) models are usually selected when picking among a finite set of models. It is similar to Akaike information criteria and partially based on likelihood function. This error function penalizes parameter count more than the AIC [40].

$$BIC = n.\ln\frac{RSS}{n} + p.\ln(n) \quad \text{(Eqn. 5)}$$

The Hannan–Quinn information criterion (HQC) is another information theory-based error function technique (Equation 7). The Hannan-Quinn information criterion is used by specialists to assess statistical model fit. This statistic is often used to compare models. Unlike the LLF, it's linked to Akaike's information criteria. Like the AIC, the HQC has a penalty function for the number of model parameters, but it is much larger because the equation contains the *ln ln* n factor [41];

$$HQC = n \times ln\frac{RSS}{n} + 2 \times p \times ln(\ln n) \quad \text{(Eqn. 7)}$$

Model suitability was assessed using BF and AF. To attain a 1 correlation between the anticipated and observed values, the Bias Factor must be 1.Bias and Accuracy Factors from predictive microbiology in food microbiology model microbial development that causes food degradation [42–49]. A fail-safe model is suggested when the Bias Factor (Equation 8) is larger than 1, while a fail-negative model is indicated when it is less than 1. Equation 9 shows that predictions with Accuracy values below 1 are less accurate.

Their susceptibility to dataset outliers is a drawback. Extreme numbers might unfairly affect computations, emphasizing isolated findings that may not represent the pattern. These factors' interpretation is context-dependent, with no universal accuracy or bias threshold. To draw significant conclusions from these elements, researchers must carefully analyze their data and curve-fitting process.

$$Bias\ factor = 10\left(\sum_{i=1}^{n} log\frac{(Pd_i/Ob_i)}{n}\right) \quad \text{(Eqn. 8)}$$

$$Accuracy\ factor = 10\left(\sum_{i=1}^{n} log\frac{|(Pd_i/Ob_i)|}{n}\right) \quad \text{(Eqn. 9)}$$

Another parameter-penalized model is MPSD. Marquardt's % standard deviation. This error function distribution follows the geometric mean error, which penalizes model parameters (Equation 10).

$$MPSD = 100\sqrt{\frac{1}{n-p}\sum_{i=1}^{n}\left(\frac{Ob_i-Pd_i}{Ob_i}\right)^2} \quad \text{(Eqn. 10)}$$

where p is the number of parameters, n is the number of experimental data, Obi is the data, and Pdi is the model prediction. The Marquardt MPSD method has limitations. Its sensitivity to initial parameter values can hinder Marquardt algorithm convergence. The convergence behavior, especially if the algorithm converges to a local minimum, may affect parameter estimates and prediction ability through percentage standard deviation. MPSD values, like other percentage measures, are scale-dependent and susceptible to outliers. With no universal fit criterion, curve-fitting procedures are difficult to evaluate without contextual information or comparison to other models.

RESULTS AND DISCUSSION

Models of substrate inhibition kinetics are essential for comprehending and regulating microbial growth at elevated substrate concentrations. In industrial processes like fermentation, these models facilitate the tuning of substrate concentrations to maximize productivity while preventing inhibition. In biofuel production, elevated substrate concentrations can impede microbial activity or completely disrupt the process. Utilizing models such as the Haldane or Aiba models, engineers can forecast and sustain the ideal substrate range, enhancing yield and minimizing waste. These models also facilitate bioremediation initiatives, wherein harmful substances are decomposed by microorganisms. Models like Luong's delineate the essential substrate concentration at which microbial degradation is optimized without inducing inhibition [50–55].

Substrate inhibition models are essential in environmental engineering for the design of effective wastewater treatment and pollution control systems. Microorganisms utilized in these systems must function within non-inhibitory substrate concentrations to effectively breakdown organic contaminants. Researchers can simulate microbial behavior and modify operational factors, like feed rate, substrate concentration, or aeration, utilizing models such as Monod, Han-Levenspiel, or Teissier-Edward. These insights enable engineers to avert system failures due to substrate inhibition, hence ensuring uninterrupted and efficient pollutant removal. Furthermore, these models are especially beneficial in mixed substrate systems, where several contaminants interact with bacteria, frequently confounding their behavior [14,17,56–58].

In addition to practical uses, these models provide substantial contributions to academic and theoretical microbiology. They offer a paradigm for examining the correlation between microbial physiology and substrate availability, particularly in nutrient-dense or hazardous situations. The Monod and Haldane models elucidate enzyme kinetics and metabolic pathways in microbial cells. Researchers get insight into how diverse microorganisms adapt to varied substrate levels by comparing models such as Luong and Aiba. This theoretical knowledge is crucial for advancing novel biotechnological applications and enhancing current systems, including bioenergy production [17,50,57,58].

Ultimately, substrate inhibition kinetics models facilitate the creation of computational tools that replicate microbial growth dynamics in practical settings. These models are essential for forecasting results in intricate contexts, such as natural ecosystems or industrial reactors. They enhance bioprocess efficiency, lower expenses, and mitigate environmental effects by offering data-driven recommendations for substrate management. Due to the significant variety in circumstances inside microbial systems, these models serve as crucial instruments for decision-making and process regulation. Their adaptability and applicability across various sectors, environmental systems, and research fields emphasize their significance in furthering both the science and practice of microbiology. These models are essential for elucidating how varying substrate concentrations affect microbial growth kinetics and biotransformation processes, which are vital in biotechnological applications such as wastewater treatment, bioremediation, and biochemical production [59,60].

As shown in **Figs. 1-10**, all ten bacterial growth models fit from very poorly for the Luong, Monod and Webb to exceptionally well for the other models. The Han-Levenspiel model was the best due to its low RMSE, BIC, HQC, and modified adjR2 values except for the MPSD and AICc stats. Furthermore, the model's AF and BF values were near to unity (**Table 2**). The experimental evidence shows that acrylamide is hazardous and hinders growth at greater dosages.

The Han-Levenspiel constants maximal degradation rate, half saturation constant, maximal substrate concentration tolerated, and curve parameter that specifies the steepness of the growth rate fall from the maximum rate are determined by u_{max}, K_s, S_m and the curve fitting parameters m and n were 16.704 h^{-1}, 3943.26 mg/L, 125.58 mg/L, 3.1469 and 0.9835, respectively, with very large confidence interval values likely due to the poor data. Other models fitted parameters also share the similar unfortune large 95% confidence interval values (results not shown), presumably due to the same reason. Future remedy includes adding more data points to improve fitting.

These biologically important results from the analysis will help guide and improve batch and field experiments. They will help researchers and environmental scientists forecast the bacterium's growth and demands when used to remediate toxicants in polluted areas. S_m, the maximum substrate concentration tolerated, is a key Han-Levenspiel model parameter. This study shows that a small number of points in curve-fitting challenge the modeling process's reliability and resilience.

The lack of data to accurately identify trends and patterns is a major drawback. With few data points, the model may overfit or underfit to determine the variable relationships. Overfitting happens when a model fits data noise rather than pattern, resulting in poor generalization to fresh data. However, underfitting occurs when the model is too simple to describe the phenomenon's complexity.

Both conditions reduce the model's prediction power and extrapolation beyond observable data points. The paucity of data points reduces statistical power to detect subtle trends or nonlinear correlations, lowering confidence in the fitted curve's accuracy and precision. Researchers must be cautious and examine alternative modeling methodologies or extra data to solve curve-fitting's intrinsic constraints of a few points. Most Han-Levenspiel model studies for xenobiotic-degrading bacteria have focused on limited xenobiotics [12,50,61–63] likely due to the high number of parameters of the model compounded with difficulty in getting more data points.

Table 2. Statistical analysis of the substrate inhibition models utilized in this study.

Model	p	RMSE	adR2	MPSD	AICc	BIC	HQC	BF	AF
Luong	4	0.0486	-5.2893	186.754	29.726	-40.491	-42.948	0.838	1.901
Yano	4	0.0103	0.8486	16.851	8.041	-62.176	-64.634	0.986	1.100
Tessier-Edward	3	0.0116	0.8177	20.810	-32.269	-60.431	-62.274	0.990	1.128
Aiba	3	0.0117	0.8197	26.694	-32.158	-60.320	-62.164	0.971	1.143
Haldane	3	0.0120	0.7555	23.686	-31.884	-60.046	-61.890	1.094	1.178
Monod	2	0.0244	-0.7209	52.847	-36.356	-50.464	-51.693	1.111	1.421
Han and Levenspiel	5	0.0083	0.8700	18.496	#DIV/0!	-66.107	-69.179	1.013	1.070
Moser	3	0.0263	-0.8987	53.993	-20.838	-49.000	-50.844	1.109	1.402
Hinshlewood	4	0.0315	-2.4418	68.225	23.644	-46.572	-49.030	1.111	1.421
Webb	4	0.0138	0.6321	27.440	12.139	-58.077	-60.535	1.094	1.179

Note: p is the number of parameters

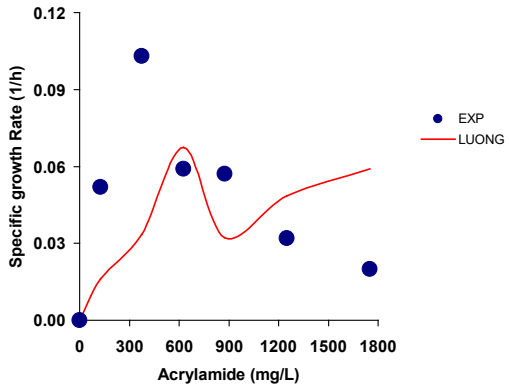

Fig. 1. Growth of *Pseudomonas* sp. strain DrY135 modeled using Luong.

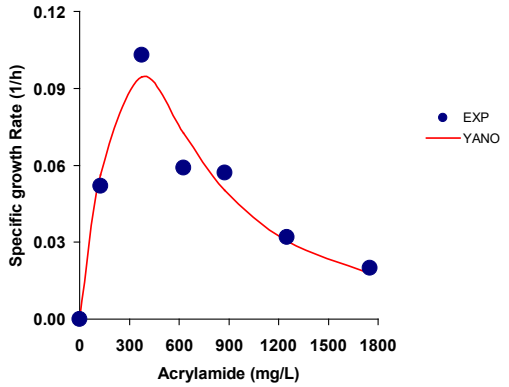

Fig. 2. Growth of *Pseudomonas* sp. strain DrY135 modeled using Yano.

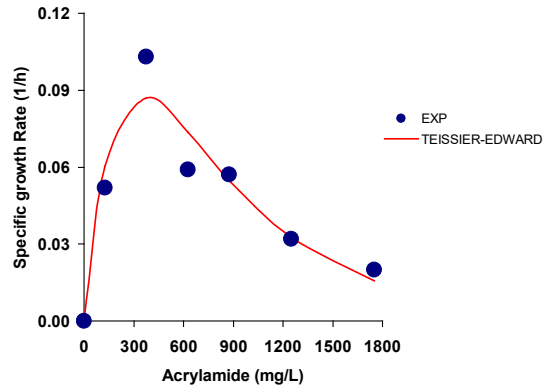

Fig. 3. Growth *of Pseudomonas* sp. strain DrY135 modeled using Teissier- Edward.

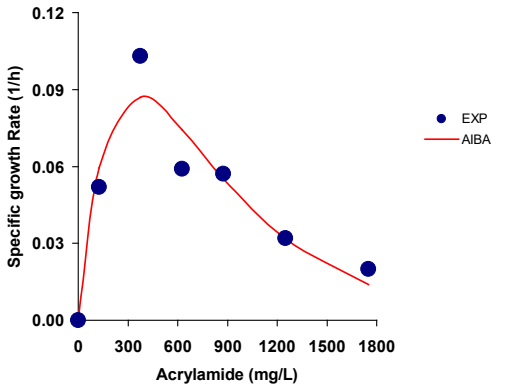

Fig. 4. Growth of *Pseudomonas* sp. strain DrY135 modeled using Aiba.

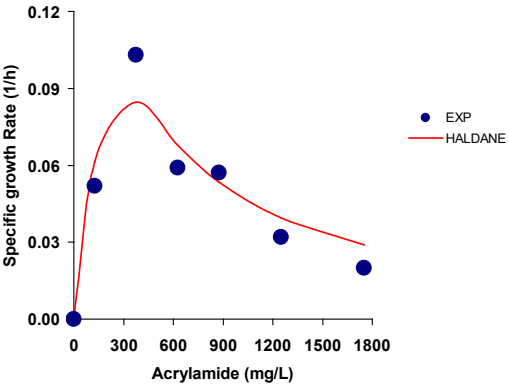

Fig. 5. Growth of *Pseudomonas* sp. strain DrY135 modeled using Haldane.

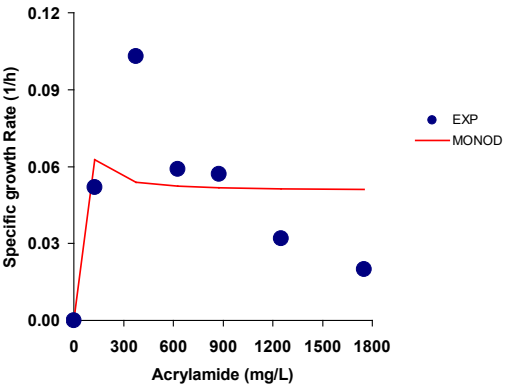

Fig. 6. Growth of *Pseudomonas* sp. strain DrY135 modeled using Monod.

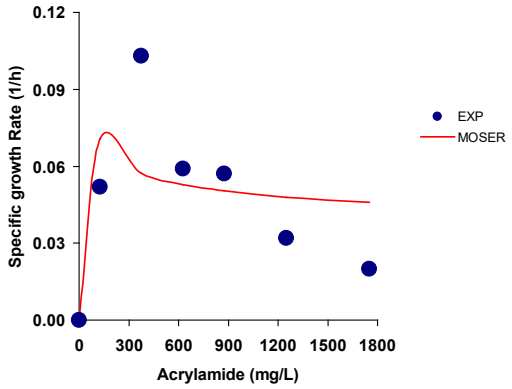

Fig. 7. Growth of *Pseudomonas* sp. strain DrY135 modeled using Moser.

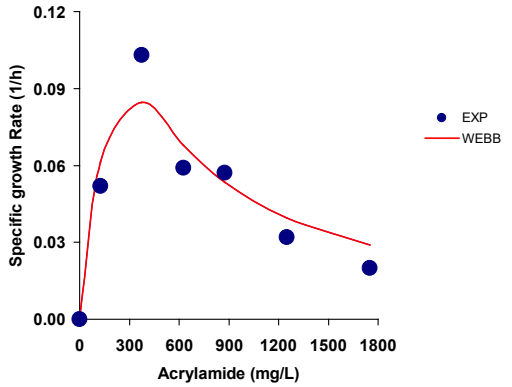

Fig. 8. Growth of *Pseudomonas* sp. strain DrY135 modeled using Webb.

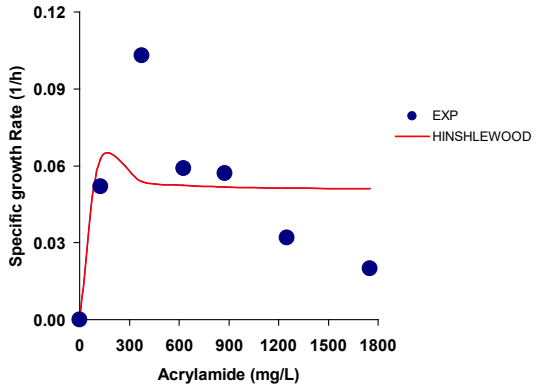

Fig. 9. Growth of *Pseudomonas* sp. strain DrY135 modeled using Hinshelwood.

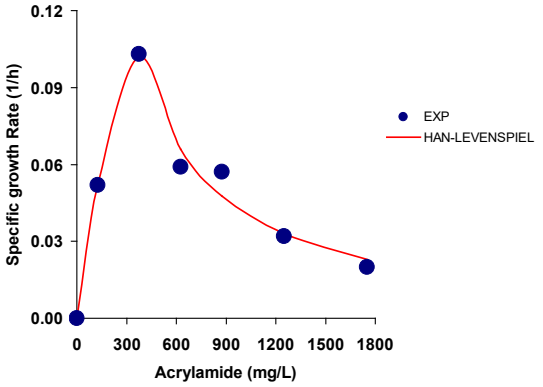

Fig. 10. Growth of *Pseudomonas* sp. strain DrY135 modeled using Han-Levenspiel.

The utilization of a substrate inhibition kinetics model is increasingly recognized as a pivotal approach for assessing the impact of toxic compounds on microbial growth or degradation rates. Traditionally, many studies have favored the Haldane or Monod models for modeling purposes. However, a select few, including this study, opt for a comprehensive modeling approach, capitalizing on the flexibility offered by alternative models. This inclusive strategy improves curve fitting results compared to popular models and represents a nuanced and thorough methodology for comprehending the intricate dynamics of microbial responses to toxic compounds. In the specific case of *Pseudomonas* sp. strain DrY135, the application of substrate inhibition kinetics provides a more detailed understanding of its behavior in acrylamide remediation, contributing to the broader field of environmental microbiology.

CONCLUSION

Substrate inhibition kinetics models offer critical insights into microbial growth and their reactions to different substrate concentrations, facilitating their use in various biotechnological domains. This study's findings underscore the significance of employing robust models like the Han-Levenspiel model, which shown enhanced efficacy in delineating the dynamics of bacterial growth suppression caused by acrylamide. The precise representation of maximal degradation rates, half-saturation constants, and tolerance thresholds provides essential information for the design and optimization of bioprocesses, including those employed in the bioremediation of hazardous contaminants. Nonetheless, the analysis highlights the difficulties associated with insufficient data points, which may undermine the reliability of curve-fitting and subsequent parameter estimates. The research highlights that augmenting data density is crucial for enhancing model accuracy and confidence intervals. This is especially critical for models with several parameters, since inadequate data may result in overfitting or underfitting, so constraining their prediction efficacy. Mitigating these restrictions would improve the application of models such as Han-Levenspiel in practical contexts, including wastewater treatment and pollution remediation.

This research emphasizes the significance of choosing suitable models matched to microbial systems and substrate dynamics. Substrate inhibition kinetics models are essential instruments for environmental and industrial microbiology, providing data-driven methodologies to enhance microbial activities. By optimizing these models via improved data acquisition and validation, researchers may more accurately forecast and influence microbial activity, aiding in sustainable solutions for environmental and biotechnological issues. The utilization of these models, together with their versatility across various systems, guarantees their ongoing significance in enhancing both scientific comprehension and practical innovation.

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