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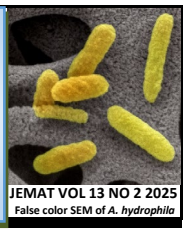

Growth Modeling of a γ-Hexachlorocyclohexane-degrading Microbial Consortium Based on Chloride Release Kinetics

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Abstract

Nonlinear growth modeling can offer a more robust approach in curve fitting exercises compared to the traditional linear regression for modelling microbial growth processes. The models utilized include Huang, Baranyi-Roberts, modified Gompertz, Buchanan-3-phase, modified Richards, modified Schnute, modified Logistics, von Bertalanffy, MMF (Morgan Mercer Flodin), and the study evaluated these primary growth models to describe a bacterial consortium growth on lindane, with growth measured indirectly by chloride release. The results show that choosing the best model using visual inspection is inadequate for distinguishing between models, as all evaluated models demonstrated acceptable fits. A statistically and information-criterion-based discriminatory approach demonstrated distinct performance disparities. The Huang model consistently demonstrated superior performance compared to competing models, characterized by the lowest error values, the highest explanatory power, favorable information criteria, and minimal systematic bias. However, the modified Richards and modified Logistics models also exhibited competitive performance under specific criteria. The MOORA multi-criteria decision-making approach was utilized to mitigate the uncertainty associated with the majority voting approach. MOORA demonstrates that the Huang model is the most robust overall, with the modified Richards and modified Logistics models following closely behind. The parameters obtained from the Huang model for the chloride release kinetics at 10 µM lindane were Lag period (d or day), $Y_{max,}$ and μ_m values of -3.132 (d), 9.235, and 0.934 (d^{-1}), respectively. The utilization of the modelling exercise yielded important parameters for future secondary modelling exercises and preliminary prediction of performance and limitations in field studies.

INTRODUCTION

Hexachlorocyclohexane (HCH) contamination remains a persistent global environmental issue arising from the large-scale application of this organochlorine insecticide during the mid-20th century [1]. HCH exists in many isomers, but only one, γ-HCH, possesses the insecticidal activity. There are two formulations of HCH, technical HCH (composed of all HCH isomers), and lindane (only γ -HCH), and both were historically used in agriculture, vector control, and commodity protection. Although regulatory bans and restrictions were introduced in many industrialized nations decades ago, residues of HCH and lindane continue to be detected in soils, sediments, and aquatic environments, particularly in regions with a legacy of intensive agricultural use or inadequate disposal practices [2,3]. Due to their chemical stability, HCH isomers persist for long periods and undergo long-range atmospheric transport, leading to their detection even in remote polar regions [4]. Accumulation of these compounds in agricultural soils facilitates entry into food chains, resulting in chronic exposure risks to wildlife and humans. Epidemiological and toxicological studies associate prolonged exposure to HCH isomers with endocrine disruption, neurotoxicity, and carcinogenic outcomes, reinforcing their classification as persistent organic pollutants of concern [5,6].

Natural attenuation of HCH in soil is typically slow and incomplete, often producing toxic chlorinated intermediates rather than full mineralization. Consequently, bioremediation has

gained prominence as a sustainable alternative to physicochemical treatment methods. Microbial degradation, particularly under aerobic conditions, offers the potential for complete dechlorination and detoxification when appropriate catabolic pathways are present [5,6]. Since the initial discovery of bacterial lindane degradation, numerous strains have been shown to harbor conserved *lin* gene clusters responsible for sequential dehydrochlorination and ring cleavage of γ-HCH, and they are predominantly within the *Sphingomonadaceae* [7].

Despite these advances, most reported isolates degrade only selected isomers, exhibit low tolerance to high substrate concentrations, or display limited activity under environmentally relevant conditions. Mixed microbial consortia have therefore attracted increasing attention, as metabolic cooperation, functional redundancy, and adaptive resilience can enhance degradation rates of recalcitrant pollutants [8,7]. However, studies examining consortium-based lindane degradation under aerobic conditions remain limited.

Growth curve modelling provides a quantitative framework for describing microbial population dynamics through the lag, exponential, and stationary phases. These primary models are essential for interpreting how bacteria respond to defined environmental and nutritional conditions before stressors or toxicants are introduced. Establishing growth behaviour in inhibitor-free systems is a critical prerequisite, as it generates baseline kinetic parameters that allow meaningful comparison when toxic substrates such as pesticides are present.

Primary growth models, including the modified Gompertz, modified Logistic, Buchanan three-phase, Baranyi–Roberts, and Richards models, have been widely applied to describe bacterial growth under controlled conditions and to extract biologically meaningful parameters such as maximum specific growth rate, lag time, and carrying capacity. These models have also been successfully used to characterise microbial growth during the degradation of recalcitrant compounds, including lindane and other organochlorine pesticides, where growth is closely coupled to dehalogenation and chloride release kinetics, as demonstrated for *Sphingomonads* and mixed consortia degrading [5,6].

In this study, predictive growth models including the modified Gompertz, modified Logistic, modified Richards, Buchanan three-phase, Baranyi–Roberts, modified Schnute, von Bertalanffy, Morgan–Mercer–Flodin, and Huang models are applied to describe the growth of a lindane-degrading bacterium. Understanding growth kinetics and dechlorination dynamics, especially through chloride release measurements, provides critical insight into metabolic performance and process optimization prior to field application.

MATERIALS AND METHODS

Nonlinear curve fitting of the bacterial growth data

CurveExpert Professional (Version 1.6) software was utilized to extract the numerical values from Figure 1a of [9]. This program uses the Marquardt method to find the smallest sum of squares of the differences between the predicted and actual values. The Marquardt algorithm iteratively changes the values of the parameters over and over again until the difference between planned and observed data is as small as possible. This makes sure that the data fits the growth curve as closely as possible in order to find the primary model that best describes how the bacterial consortia grow in lindane.
(**Table 1**).

Table 1. Mathematical modeling of growth on lindane by a bacterial consortium.

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

Finding the best model based on visual observation is never accurate, and hence, a better method is to use statistical discriminatory methods. The tests utilized in this study were the Marquardt's percent standard deviation (MPSD) [10–12], HQ (Hannan and Quinn's Criterion) [13], Bias Factor (BF), Accuracy Factor (AF) [14], root-mean-squared error (RMSE), adjusted coefficient of determination (R^2) [15], corrected Akaike Information Criterion (AICc) [16,17], and Bayesian Information Criterion (BIC) [18]. In general, n is the total number of observations, *Obi* and *Pdi* are the predicted and observed values, and p is the total number of parameters of the model [19].

RMSE was calculated using the following formula;

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

BF and AF were calculated using the following formula;

$$Bias\ factor = 10\left(\sum_{i=1}^{n} log\frac{(Pd_i/Ob_i)}{n}\right) \quad \text{(Eqn. 2)}$$

$$Accuracy\ factor = 10\left(\sum_{i=1}^{n} log\frac{|(Pd_i/Ob_i)|}{n}\right) \quad \text{(Eqn. 3)}$$

AICc was calculated using the following formula;

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

BIC was calculated using the following formula;

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

HQC was calculated using the following formula;

$$HQC = nIn\left(\frac{RSS}{n}\right) + 2k\ln(\ln n) \quad \text{(Eqn. 6)}$$

Adjusted coefficient of determination (R^2) was calculated using the following formula;

$$Adjusted\ (R^2) = 1 - \frac{RMS}{S_Y^2} \quad \text{(Eqn. 7)}$$

$$Adjusted\ (R^2) = 1 - \frac{(1-R^2)(n-1)}{(n-p-1)} \quad \text{(Eqn. 8)}$$

MPSD was calculated using the following formula;

$$MPSD = 100\sqrt{\frac{1}{n-p}\sum_{i=1}^{n}\left(\frac{Ob_i - Pd_i}{Ob_i}\right)^2} \quad \text{(Eqn. 9)}$$

Application of Multi-Objective Optimization by Ratio Analysis (MOORA) in Modeling

Often, a non-unanimous result for the error function means it is more difficult to choose the best model. A method to overcome this issue is the modeling exercise's multi-criteria decision-making (MCDM), specifically MOORA, which simultaneously evaluates all of the error functions. This approach makes it easier to identify the ideal model [20,21]. Initially, a standardization of the decision matrix was carried out through a normalization approach as the units and magnitudes of each of the error functions usually differ.

$$X'_{ij} = \frac{X_{ij}}{\sqrt{\sum_{i=1}^{n} X_{ij}^2}} \quad \text{(Eqn. 10)}$$

Where X_{ij} is the original value of the j^{th} metric for the i^{th} model, and X^{i}_{ij} is the normalized value**.**

Ratio System Analysis

After that, a ratio method was used to figure out the combined normalized numbers. We used the following formula that sums all of the beneficial criteria (adjR2) and then subtracts the values of the non-beneficial criteria (the other error functions) or error functions that need to be kept to a minimum. Here, we use a cost function for the error function bias factor (BF) or CBF=|1−BF| , whilst the cost function for the error function accuracy factor (AF) or CAF=AF−1.

$$Y_i = \sum_{beneficial} X'_{ij} - \sum_{non-beneficial} X'_{ij} \quad \text{(Eqn. 11)}$$

Where Y_i is the final score for the i^{th} model

Since there will be a certain criteria that are more important than others, it is best to use weighted ratios. Exhaustive literature search shows no evidence to support the suggestion to use Weighted Ratios in the primary models as it is inconclusive and probably not accurate to assign importance of one model above another. Ultimately, the models are ranked in order based on their overall performance ratings, with the higher score models ranking higher. This method allows the comparison or selection for the best kinetic models be done in a systematic and objective way, which helped us find the one that worked best for all of our performance criteria.

RESULTS AND DISCUSSION

For many years, scientists have used linear regression to find the slope of a growth curve or the parameters of a xenobiotic transformation after manually estimating the part of the curve that is almost linear. A better way to do this would be to use a nonlinear regression growth model to describe the whole dataset and then use the model to estimate the values of the μ_m, λ, and A [22]. The parameter μ_m can then be used for further secondary modeling like Monod, Haldane, Aiba, and Teissier [23,24]. All of the models appear to fit the original data (**Fig. 1**) adequately (**Figs. 2** to **10**), and it is quite difficult to choose the best model based on visual observation.

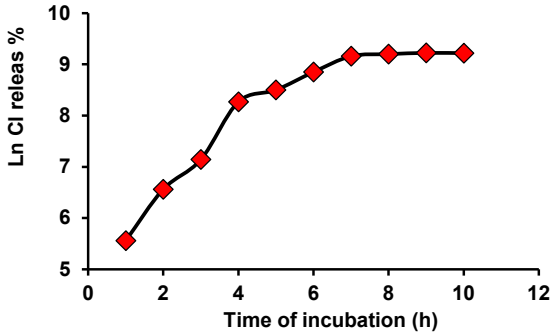

Fig. 1. Natural logarithm converted data on the percentage chloride release from the γ-hexachlorocyclohexane-degrading microbial consortia.

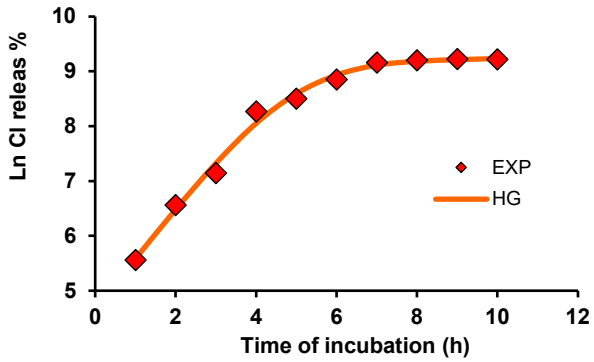

Fig. 2. Modeling of the chloride release kinetics of the γ-hexachlorocyclohexane-degrading microbial consortia based on the Huang model.

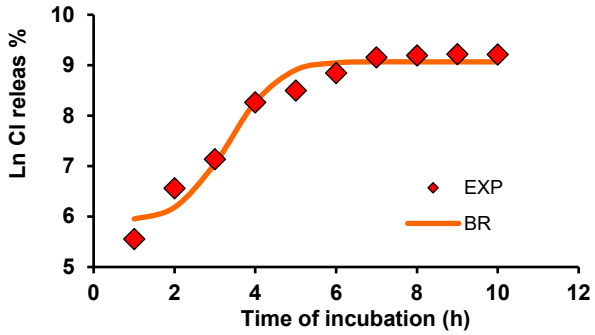

Fig. 3. Modeling of the chloride release kinetics of the γ-hexachlorocyclohexane-degrading microbial consortia based on the Baranyi-Roberts model.

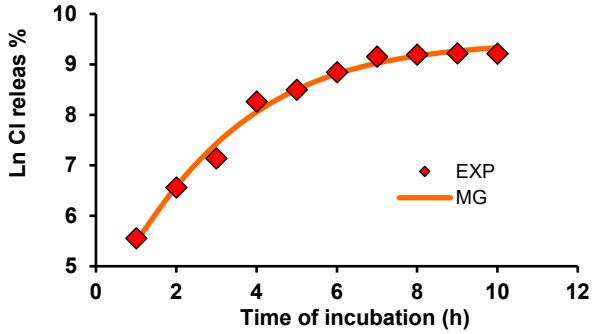

Fig. 4. Modeling of the chloride release kinetics of the γ-hexachlorocyclohexane-degrading microbial consortia based on the modified Gompertz model.

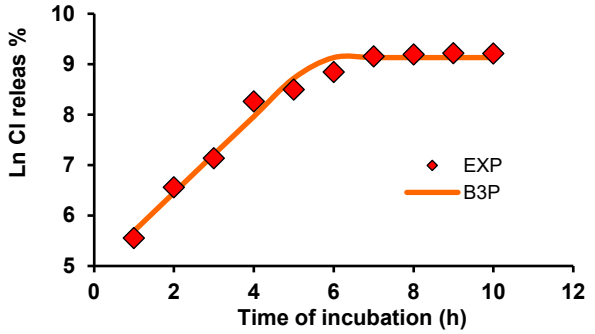

Fig. 5. Modeling of the chloride release kinetics of the γ-hexachlorocyclohexane-degrading microbial consortia based on the Buchanan-3-phase model.

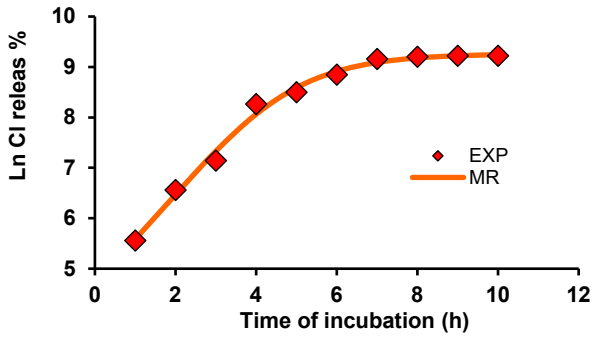

Fig. 6. Modeling of the chloride release kinetics of the γ-hexachlorocyclohexane-degrading microbial consortia based on the modified Richards model.

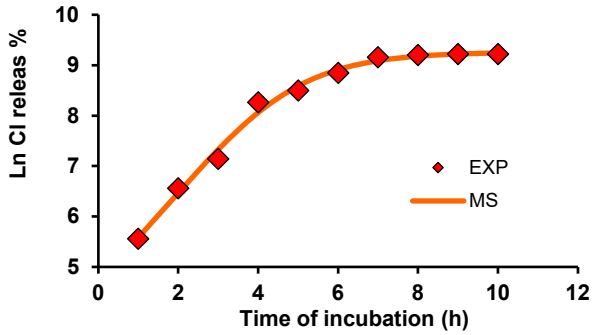

Fig. 7. Modeling of the chloride release kinetics of the γ-hexachlorocyclohexane-degrading microbial consortia based on the modified Schnute model.

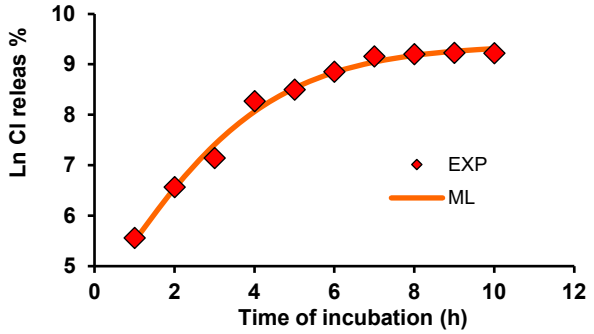

Fig. 8. Modeling of the chloride release kinetics of the γ-hexachlorocyclohexane-degrading microbial consortia based on the modified Logistics model.

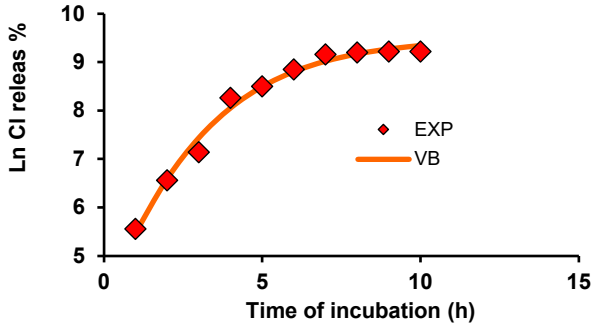

Fig. 9. Modeling of the chloride release kinetics of the γ-hexachlorocyclohexane-degrading microbial consortia based on the von Bertalanffy model.

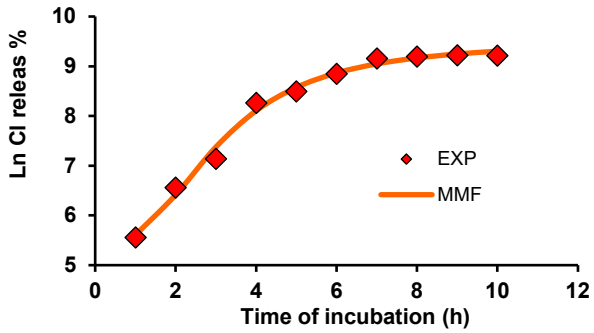

Fig. 10. Modeling of the chloride release kinetics of the γ-hexachlorocyclohexane-degrading microbial consortia based on the MMF model.

Table 2. Statistical analysis of the various fitted models.

Model	*p*	RMSE	R2	adR2	AF	BF	AICc	BIC	HQC	MPSD
Huang	4	0.102	0.993	0.990	1.007	1.000	-43.138	-58.082	-60.875	10.197
Baranyi-Roberts	4	0.242	0.960	0.942	1.019	1.001	-18.907	-33.851	-36.644	24.226
modified Gompertz	3	0.288	0.940	0.922	1.013	1.000	-19.770	-30.297	-32.392	28.817
Buchanan-3-phase	3	0.159	0.981	0.976	1.013	1.000	-36.401	-46.929	-49.023	15.910
modified Richards	4	0.102	0.993	0.990	1.007	1.000	-43.019	-57.963	-60.755	10.240
modified Schnute	4	0.230	0.965	0.950	1.007	1.000	-20.417	-35.361	-38.154	22.954
modified Logistics	3	0.113	0.991	0.988	1.008	1.000	-46.038	-56.566	-58.660	11.277
von Bertalanffy	3	0.128	0.988	0.984	1.009	1.000	-42.441	-52.968	-55.063	12.823
MMF	4	0.115	0.967	0.952	1.009	1.000	-39.767	-54.710	-57.503	11.501

Note: *p* is parameter

The modelling results show a relatively good fit for all of the primary growth models utilized in this study. The Huang model did the best overall because it did the best on most of the error-based and information-based criteria (**Table 2**). It exhibited the lowest SSE (0.1040), MSE (0.0104), RMSE (0.1020), and MPSD (10.20), the highest R^2 (0.9931) and adjusted R^2 (0.990). It also had the best BIC (-58.08) and HQC (-60.87) scores, and AF and BF were closest to unity (AF 1.01, BF 1.00), which indicates minimal systematic bias. The modified Richards model came in a very close second, with R^2 and *adjusted* R^2 values that were close (0.9931 and 0.990) and error values that were also very low (SSE 0.1049, RMSE 0.1024, MPSD 10.24).

However, the values for the information criterion were a little weaker than Huang's. The modified Logistics model was also competitive and had the lowest value for AICc (-46.04), indicating its preference according to that standard. However, it did not do better than Huang on other error functions (SSE, RMSE, MPSD) or on BIC and HQC. The modified Gompertz model was the worst because it had the largest values of errors (SSE 0.9134, RMSE 0.2882, MPSD 28.82) and the lowest values for R^2 0.9398, and *adjusted*R^2 0.922). This means it was the least accurate to fit the growth curve measured as chloride release.

The method of selecting the optimal growth model through majority voting based on error functions and information criteria usually introduces uncertainty, as the results can be unanimous with one model exhibiting the best values for a selected error function and the rival models offer better values for the remaining error functions. The relationships among SSE, MSE, and RMSE, all of which are mathematically related, can indicate that a model exhibiting strong performance in one metric typically demonstrates favorable results in the other error functions. This can potentially lead to an overemphasis on a singular aspect of model fit. Moreover, the classical error functions prioritize distinct objectives, including how closely the predicted data obey the observed data, while modern error functions based on information criteria such as AICc, BIC, and HQC impose varying penalties for the number of parameters a model has.

A model like modified Logistics may demonstrate superiority under AICc, whereas an alternative model may perform better under BIC or RMSE (**Table 2**). Minor numerical differences can lead to a practical conclusion of nonsignificance in the absence of confidence intervals, residual diagnostics, or validation using independent data. MOORA, by consolidating multiple criteria into a singular composite score, mitigates this ambiguity following normalization and weighting (**Table 3**). This approach filters trade-offs and thwarts "double counting" of closely related error function values. The MOORA ranking indicates that the Huang model is the highest rated overall (score 1.49351, rank 1). This is closely followed by the modified Richards model (1.48904, rank 2) and the modified Logistics model (1.45304, rank 3). The Huang model can offer a continuous and biologically precise depiction of bacterial growth throughout the phases of the Lag, exponential, and stationary. The use of classical first-order kinetics can effectively describe exponential growth parts; however, they cannot model the Lag and stationary phases. To address this limitation, many nonlinear growth kinetic models incorporate a maximum carrying capacity, which facilitates a smooth transition from the exponential growth phase to the stationary phase.

Table 3. MOORA ranking of the error functions.

No	Model	MOORA Score	Rank
1	Huang	1.49351	1
5	modified Richards	1.48904	2
7	modified Logistics	1.45304	3
9	MMF	1.34535	4
8	von Bertalanffy	1.31077	5
4	Buchanan-3-phase	1.05178	6
6	modified Schnute	0.47606	7
2	Baranyi-Roberts	0.38590	8
3	modified Gompertz	0.15704	9

Huang and colleagues integrate the lag and growth phases into a singular continuous equation and proposed a unified framework. The integration of a transition function that alleviates abrupt and discontinuous phase switching is the key to the success of this model. Near the lag time, the transition function incrementally shifts from zero to one. This thereby maintains mathematical continuity and biological interpretation. One of the hallmarks of the Huang model is it can demonstrate robustness despite the unavailability of data governing the lag phase data, and convergence in nonlinear regression can occur relatively rapidly and remains stable. Growth proceeds according to the classical exponential kinetics after the completion of the lag phase, until the stationary phase is attained.

The integration of attributes, including continuity, interpretability, and numerical stability, is a testament to the Huang model's applicability in analyzing microbial growth and also for predictive uses in bioremediation and environmental biotechnology. The parameters obtained from the Huang model for the chloride release kinetics at 10 µM lindane is Lag (d), Y_{max} and μ_m values of -3.132 day (95% C.I too wide), 9.235 (95% C.I. from 9.043 to 9.427), and 0.934 d^{-1} (95% C.I. from0.710 to 1.158). In the original published works [9], no modelling was carried out. The utility of the Huang model to model growth processes is evident in its use in modelling biological processes in the microorganisms *Cronobacter sakazakii*, *Escherichia coli* O157:H7, *Pseudomonas* spp., *Listeria monocytogenes*, *Salmonella* spp., and *Streptomyces lividans* [25–31]. The model's applicability is its having excellent fitting capability, and its lag period predictive ability, with most of the model parameters exhibiting biological meaning [32,33].

CONCLUSION

This study demonstrates the applicability of nonlinear primary growth models to provide a more comprehensive and objective framework for analyzing a microbial consortium's growth on lindane biodegradation. It is discovered that the various models, although adequately aligned with the experimental data, have issues when over-reliance on individual error functions or visual assessments introduces uncertainty due to overlapping and correlated performance metrics. The MOORA method reduced this ambiguity by its ability to integrate these various criteria into one unified ranking. MOORA designates the Huang model as the best model. The Huang model's continuous structure, its ability to provide an explicit representation of the lag phase, and its numerical robustness have enabled biologically relevant interpretations to be obtained despite some constraints in the lag-phase data. The estimated parameters obtained from the modelling exercise for chloride release kinetics allow future secondary inhibition models integration. The results in this study demonstrate the importance of a combination of robust nonlinear modeling with multi-criteria decision analysis for obtaining the best growth models. This dual approach method can improve predictive accuracy and can support the systematic design and enhancement of bioremediation strategies in field studies on environments contaminated by pesticides.

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