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Research article

Mathematical modelling of the degradation kinetics of *Bacillus cereus* grown on phenol

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ABSTRACT

The mathematical modelling of the effect of substrate concentration on growth rate of bacteria is crucial in the understanding of the many phenomena in xenobiotics biodegradation. The rate constants obtained from this modeling allow the mathematical prediction of growth parameters. We remodelled a previously published work on phenol degradation by *Bacillus cereus* MTCC 9817 strain AKG1 using several more growth kinetic models such as Monod, Teissier, Andrews and Noack, Hinshelwood, Moser, Aiba, Webb (Edward), Yano and Koga, Han and Levenspiel and Luong and evaluated the accuracy of the fitted model using statistical analysis such as Root Mean Square (RMSE), adjusted Coefficient of Determination (R^2), corrected Akaike Information Criterion (AICc), Bias Factor, Accuracy Factor and F-test. The calculated values for the best model- Luong's such as maximal degradation rate, half saturation constant for maximal degradation, maximal concentration of substrate tolerated and curve parameter that defines the steepness of the growth rate decline from the maximum rate, symbolized by q_{max}, K_s, S_m, and n were 0.755 hr^{-1}, 925.8 mg/L, 1859.3 mg/L and 0.329, respectively. The true value of q_{max} determined as the value where the gradient for the slope is zero was 0.093 h^{-1} at 500 mg/L phenol. The results indicate that the exhaustive use of mathematical models on available published results could gleam new optimal models that can provide new knowledge on the way toxic substance inhibit growth rate in microbes.

INTRODUCTION

Quantitative experimental data is required for the design and optimization of biological transformation processes. A variety of mathematical models have been proposed to describe the dynamics of metabolism of compounds exposed to pure cultures of microorganisms or microbial populations of natural environment. The relation between the specific growth rate (μ) of a population of microorganisms and the substrate concentration (s) is a valuable tool in biotechnology. the Monod equation has been widely used to describe growth-linked substrate utilization rate [1–3]. However, when a substrate exhibits inhibition towards its own biodegradation, the original Monod model could not be used. In this case, its derivatives that have new constants that provided corrections for substrate have been devised instead. a variety of microbial growth of for this work biodegradation kinetic model available is shown in table 1. The generalization of the use of the Haldane model in literature to model substrate inhibition to growth or degradation rate is numerous literatures. This is despite the fact, that for a single substrate-inhibiting compound such as phenol, several other models have been demonstrated to be more accurate. for instance, aside from the predominantly reported Haldane model [4], several other different models have been found to be optimal such as Luong [5,6] and Edward [7]. Hence, the use of extensive models available could replace the Haldane in some circumstances. without actually fitting these other models to the available growth or degradation rate data and proper statistical evaluation, the exclusive use of the Haldane model should not be used liberally.

Hence, the objective of this work is to evaluate similarities and differences between the models using published available data for further more comprehensive modeling and to deal with the question of which model(s) can be used, on the basis of statistical reasoning. This should give new data and results that could spurn and reveal new information and improvement in the works already done by researchers.

MATERIALS AND METHODS

Acquisition of data

In order to process the data, the graph showing the degradation rate against substrate phenol concentration [15] for *Bacillus cereus* MTCC 9817 strain AKG1 in figure 3 were electronically processed using Webplotdigitizer 2.5 [16] which helps to digitize scanned plots into table of data with good enough precision [17].

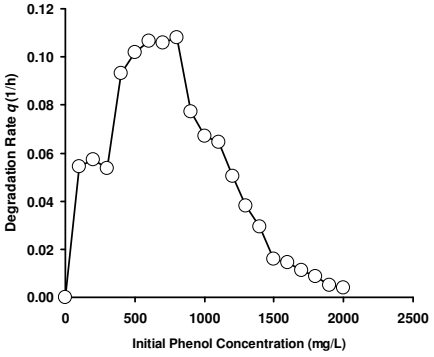

Figure 1. Replotted data of the degradation rate against substrate phenol concentration for *Bacillus cereus* MTCC 9817 strain AKG1.

Table 1. Various mathematical models developed for degradation kinetics involving substrate inhibition.

Author	Degradation rate	Author
Monod	$q_{\max}\frac{S}{K_s+S}$	[8]
Haldane	$q_{\max}\frac{S}{S+K_s+\frac{S^2}{K_i}}$	[9]
Teissier	$q_{\max}\left(1-\exp\left(-\frac{S}{K_i}\right)-\exp\left(\frac{S}{K_s}\right)\right)$	[10]
Aiba	$q_{\max}\frac{S}{K_s+S}\exp(-KP)$	[11]
Yano and koga	$\frac{q_{\max}S}{S+K_s+\left(\frac{S^2}{K_1}\right)\left(1+\frac{S}{K}\right)}$	[12]
Han and levenspiel	$q_{\max}\left[1-\left(\frac{S}{S_m}\right)\right]^n\left[\frac{S}{S+K_s\left(1-\frac{S}{S_m}\right)^m}\right]$	[13]
Luong	$q_{\max}\frac{S}{S+K_s}\left[1-\left(\frac{S}{S_m}\right)^n\right]$	[14]

note:
q_{max} maximal degradation rate (h^{-1})
k_s half saturation constant for maximal degradation (mg/l)
s_m maximal concentration of substrate tolerated and (mg/l)
m, n, k curve parameters
s substrate concentration (mg/l)
p product concentration (mg/l)

Fitting of the data

The nonlinear equations were fitted to growth data by nonlinear regression with a Marquardt algorithm that minimizes sums of square of residuals using Curveexpert professional software (version 1.6). This is a search method to minimize the sum of the squares of the differences between the predicted and measured values. The program automatically calculates starting values by searching for the steepest ascent of the curve between four datum points (estimation of μ_{max}), by intersecting this line with the x axis (estimation of λ), and by taking the final datum point as estimation for the asymptote (a). The huang's model needs to be solved numerically as it is a differential equation. The differential equation was solved numerically using the Runge-Kutta method. A differential equation solver (ode45) in Matlab (version 7.10.0499, the Mathworks, Inc., Natick, MA) was used to solve this equation.

Statistical analysis

To decide whether there is a statistically substantial difference between models with different number of parameters, in terms of the quality of fit to the same experimental data was statistically assessed through various methods such as the root-mean-square error (RMSE), adjusted coefficient of determination (R^2), bias

factor (BF), accuracy factor (AF), corrected AICc (akaike information criterion) and f-test [18].

The RMSE was calculated according to eq. (2), where pd_i are the values predicted by the model and ob_i are the experimental data, n is the number of experimental data, and p is the number of parameters of the assessed model. It is expected that the model with the smaller number of parameters will give a smaller RMSE values.

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

In linear regression models the coefficient of determination or r^2 is used to assess the quality of fit of a model. However, in nonlinear regression where difference in the number of parameters between one models to another is normal, the adoption of the method does not readily provides comparable analysis. Hence an adjusted R^2 is used to calculate the quality of nonlinear models according to the formula where RMS is Residual Mean Square and S_y^2 is the total variance of the y-variable.

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

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

The Akaike Information Criterion (AIC) provides a means for model selection through measuring the relative quality of a given statistical model for a given set of experimental data [19]. AIC handles the trade-off relating to the goodness of fit of the model as well as the complexity of the model. It is actually established on information theory. The method provides a relative approximation of the information lost for each time a given model is utilized to represent the process that creates the information or data. For an output of a set of predicted model, the most preferred model would be the model showing the minimum value for AIC. This value is often a negative value, with for example; an AICc value of -10 more preferred than the one with -1. The equation incorporates number of parameters penalty, the more the parameters, the less preferred the output or the higher the aic value. Hence, AIC not merely rewards goodness of fit, but in addition does not encourage using more complicated model (overfitting) for fitting experimental data. Since the data in this work is small compared to the number of parameter used a corrected version of Aic, the Akaike Information Criterion (AIC) with correction or AICc is used instead. The AICc is calculated for each data set for each model according to the following equation;

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

Where n is the number of data points and p is the number of parameters of the model. The method takes into account the change in goodness-of-fit and the difference in number of parameters between two models. for each data set, the model with the smallest AICc value is highly likely correct [20].

The f-test is a statistic test used to find the most significant model between available predicted curve-fitting models. The analysis procedure includes selecting the model with the smallest rss among all the models with the same or different number of fitting parameters followed by comparing the relative value of the f-ratio. In the event the f-ratio of the two models surpasses the upper quartile, the better complicated model is accepted as statistically significant [20]. Equation 5 is for models with same number of parameters while equation 6 is for models with different number of parameters;

$$F = \frac{SS_1}{SS_2} \quad (5)$$

$$F = \frac{(SS_1 - SS_2)/(df_2 - df_1)}{SS_2 / df_2} \quad (6)$$

Accuracy factor (AF) and bias factor (BF) to test for the goodness-of-fit of the models as suggested by Ross [21] were also used. The bias factor equal to1 indicate a perfect match between predicted and observed values. For microbial growth curves or degradation studies, a bias factor with values < 1 indicates a fail-dangerous model while a bias factor with values > 1indicates a fail-safe model. The accuracy factor is always ≥ 1, and higher AF values indicate less precise prediction.

$$\text{Bias factor} = 10^{\left(\sum_{i=1}^{n}\log\frac{(Pd_i/Ob_i)}{n}\right)} \quad (7)$$

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

RESULTS AND DISCUSSION

The results of the curve fitting are shown in figures 2 to 6. models such as Webb, Hinshelwood, Andrews and Noack, and Han and Levenspiel failed to fit the experimental data and were omitted. All of the other models tested with the exception of the Monod model gave reasonably good fitting based on visual observation.

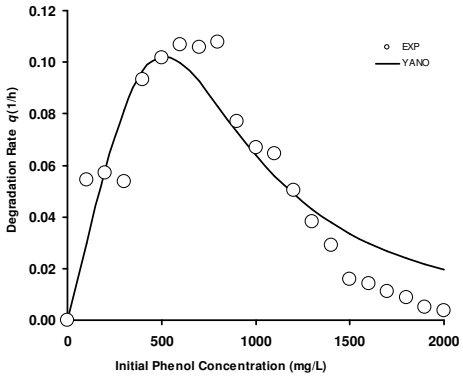

Figure. 2. Fitting experimental data with the Yano model.

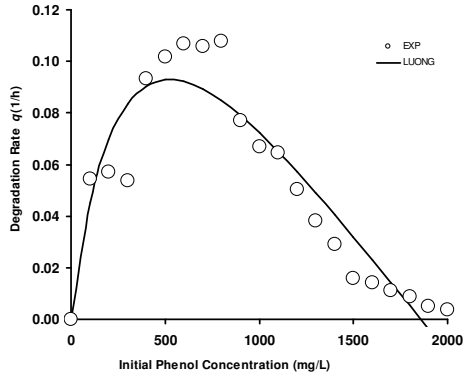

Figure. 3. Fitting experimental data with the Luong model.

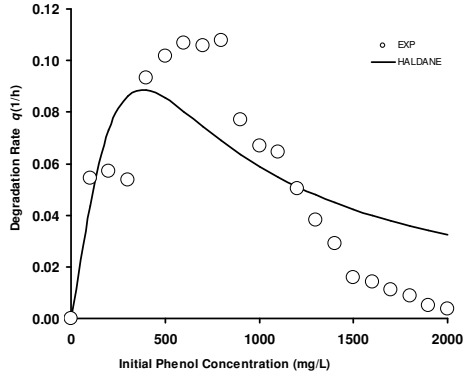

Figure. 4. Fitting experimental data with the Haldane model.

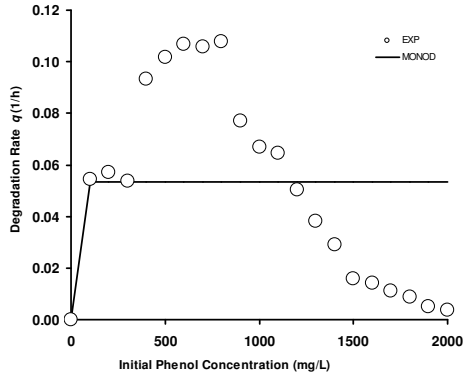

Figure. 5. Fitting experimental data with the Monod model.

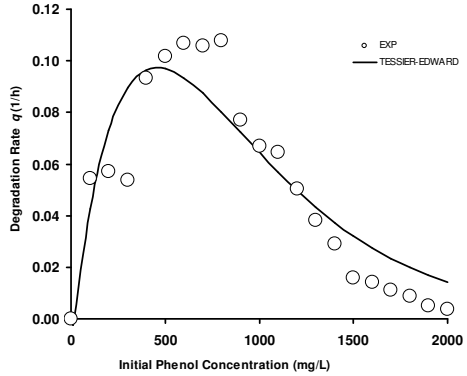

Figure. 6. Fitting experimental data with the Teissier-Edward model.

The accuracy and statistical analysis of the six kinetic models used shows that the best model was Luong with low values for RMSE and AICc, highest adjusted r^2 values, f-test and with bias factor and accuracy factor nearest to unity (1.0) (table 2). The calculated value for the Luong's constants maximal degradation rate, half saturation constant for maximal degradation, maximal concentration of substrate tolerated and curve parameter that defines the steepness of the growth rate decline from the maximum rate symbolized by q_{max}, k_s, s_m, and n were 0.755 hr^{-1}, 925.8 mg/l, 1859.3 mg/l and 0.329, respectively. The best model according to Banerjee and Ghoshal [15] where the experimental data for this work was procured is Edward (Tesssier) with calculated maximal degradation rate, half saturation constant for maximal degradation, inhibition constant symbolized by q_{max}, k_s and k_i, are 0.4701 h^{-1}, 407 mg/l and 431.3 mg/l, respectively. The same constants for the same model obtained in this work were quite similar with q_{max}, k_s and k_i values obtained were 0.993 h^{-1}, 514.3 mg/l and 391.3 mg/l, respectively. It needs to be cautioned that the q_{max} value obtained based on curve fitting interpolation is not the true value as the true q_{max} should be where the gradient for the slope is zero and in this case (Luong) the value was approximately 0.093 h^{-1} at 500 mg/l phenol (figure 3).

Table 2. Statistical analysis of kinetic models.

Model	p	RMSE	R^2	adR^2	AICc	BF	AF
Luong	4	0.013	0.881	0.851	-164.2	1.07	1.22
Yano	4	0.015	0.799	0.749	-159.3	1.15	1.30
Tessier-Edward	3	0.015	0.792	0.755	-163.1	1.29	1.45
Aiba	3	0.015	0.780	0.741	-163.0	1.30	1.45
Haldane	3	0.023	0.072	-0.091	-145.8	1.47	1.78
Monod	2	0.035	-7.584	-8.538	-131.5	1.44	2.15

note:
sse sums of squared errors
RMSE root mean squared error
p no of parameters
R^2 coefficient of determination
adR^2 adjusted coefficient of determination
AICc corrected Akaike Information Criterion
BF bias factor
AF accuracy factor

Most of the studies concerning substrate inhibition on microbial growth are carried out using toxic substrate such as aromatic and halogenated hydrocarbons [22,23], and hence it can be deducted that at high concentration growth rate will be severely affected and the normal use of the Monod model will fail.

There were other models for describing substrate inhibition kinetics developed during this period such as the discontinuous models of Wayman and Tseng [24]. The reason for the development of the discontinuous model is the previous models developed such as Haldane, Andrews and Noack, and Webb can describe inhibitory effect on microbial growth but could not explain or adequately model for certain situations where the growth rate completely ceased or becoming zero at very high substrate concentration. however, the discontinuous fitting profile of the Wayman and Tseng model is a major drawback [25]. A continuous version of the above models developed by Luong have found popular support due to its close agreement to experimental data in a number of cases [5,6,26] including this one. A central attraction of the Luong model is its ability to successful predicting

the value of s_m, the maximum substrate concentration above which growth is completely inhibited.

CONCLUSION

Both growth and degradation kinetics of bacteria can be modeled using various models available in the literature. Literature survey has shown that for the same compound, various models have been found optimum in different systems and hence a comprehensive modeling exercise was carried out on available published works to demonstrate this observation. In this work, we demonstrated based on statistical analysis that the Luong model is a better model than the Edward (Teissier) in fitting the degradation kinetics data from *Bacillus cereus* MTCC 9817 strain akg1. We predicted that many existing published models in the literature could be better modelled using the various kind of growth or degradation models available instead of the ubiquitous Haldane model for instance.

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