Research article

Mathematical Modeling of the Growth Kinetics of *Bacillus* sp. on Tannery Effluent Containing Chromate

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ABSTRACT

Kinetic equations, which describe the activity of an enzyme or a microorganism on a particular substrate, are crucial in understanding many phenomena in biotechnological processes. They allow the mathematical prediction of growth parameters important for identifying key parameters for controlling growth. We remodeled the published work on chromate reduction by *Bacillus* sp. (JUBTCR3) using several more growth kinetic models such as Monod, Teissier, Andrews and Noack, 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 value for the Haldane constants in this work such as maximal growth rate, half saturation constant and half inhibition constant rate symbolized by u_{max}, k_s, and k_i, were 0.07 hr^{-1}, 17.4 mg/dm^3 and 102.95 mg/dm^3, respectively. The true u_{max} where the gradient for the slope is zero for the Haldane model was approximately 0.037 h^{-1} at 42 mg/dm^3 chromate. the results indicate that the exhaustive use of mathematical models on available published results could support published results using comparative analyses of more models backed by statistical analyses that can provide new knowledge on the way toxic substance inhibit growth rate in microbes.

INTRODUCTION

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 relationship between the specific growth rate (μ) and the substrate concentration (s) is a valuable tool in predictive biotechnology. The most widely used model; the Monod equation has been widely used to describe growth-linked substrate utilization rate [1,2]. However, when a substrate exhibits inhibition towards its own biodegradation or bioreduction, 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 reduction kinetics 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 [3], several other different models have been found to be optimal such as Luong [4,5] and Edward [6]. 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 growth rate against the substrate chromate published by Samanta et al. [15] by *Bacillus* sp. (JUBTCR3) on figure 4 was electronically processed using Webplotdigitizer 2.5 [16] which helps to digitize scanned plots into table of data with good enough precision [17].

Fitting of The Data
The non-linear 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.

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].

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

Author	Reduction Rate	Author
Monod	$q_{max}\dfrac{S}{K_s+S}$	[7,8]
Haldane	$q_{max}\dfrac{S}{S+K_s+\dfrac{S^2}{K_i}}$	[9]
Teissier	$q_{max}\left(1-\exp\left(-\dfrac{S}{K_i}\right)-\exp\left(\dfrac{S}{K_s}\right)\right)$	[10]
Aiba	$q_{max}\dfrac{S}{K_s+S}\exp(-KP)$	[11]
Yano And Koga	$\dfrac{q_{max}S}{S+K_s+\left(\dfrac{S^2}{K_1}\right)\left(1+\dfrac{S}{K}\right)}$	[12]
Han And Levenspiel	$q_{max}\left[1-\left(\dfrac{S}{S_m}\right)\right]^n\left[\dfrac{S}{S+K_s\left(1-\dfrac{S}{S_m}\right)^m}\right]$	[13]
Luong	$q_{max}\dfrac{S}{S+K_s}\left[1-\left(\dfrac{S}{S_m}\right)^n\right]$	[14]

Note:
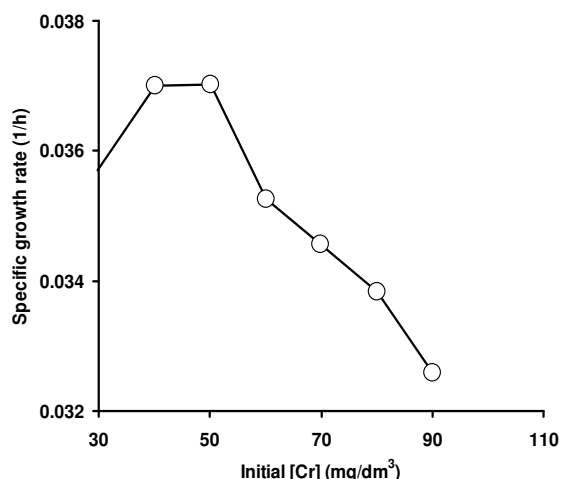|	
q_{max}	Maximal Reduction Rate (h^{-1})
k_s	Half Saturation Constant For Maximal reduction (mg/dm^3)
S_m	Maximal Concentration of Substrate Tolerated (mg/dm^3)
m, n, k	Curve Parameters
s	Substrate Concentration (mg/dm^3)
p	Product Concentration (mg/dm^3)

Figure 1. Replotted data of the growth rate against the substrate chromate concentration for *Bacillus* sp. (JUBTCR3).

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.

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

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 model 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.

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

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

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;

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

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 [18].

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 [18]. Equation 5 is for models with same number of parameters while equation 6 is for models with different number of parameters;

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

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

Accuracy factor (AF) and bias factor (BF) to test for the goodness-of-fit of the models as suggested by Ross [20] 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.

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

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

RESULTS AND DISCUSSION

The results of the curve fitting are shown in figures 2 to 5. Models such as Monod, Teissier, Andrews and Noack, and Han and Levenspiel failed to fit the experimental data and were omitted. All of the other models tested gave reasonably good fitting based on software output and by visual observation. The accuracy and statistical analysis of the six kinetic models used shows that the best model was Haldane with the lowest value for RMSE, AICc and the highest value for adjusted R^2. The AF and BF values were also excellent for Haldane with their values were the closest to 1.0 (Table 2). F-test analysis showed that the likelihood that the Haldane model is better than Yano, Luong and Aiba were 97.6, 98.3 and 69.25%, respectively; indicating in overall the Haldane model was the best. The original author for this data also concludes that the Haldane model is the best

The calculated value for the Haldane constants in this work such as maximal growth rate, half saturation constant and half inhibition constant rate symbolized by u_{max}, k_s, and k_i, were 0.07 hr^{-1}, 17.4 mg/dm^3 and 102.95 mg/dm^3, respectively. The calculated values for the Haldane constants in the work of Samanta et al. [15] for the growth rate of *Bacillus* sp. (JUBTCR3) such as u_{max}, k_s, and k_i, were 0.056 hr^{-1}, 9.43 mg/dm^3 and 305.29 mg/dm^3, respectively.

It needs to be cautioned that the u_{max} value obtained based on curve fitting interpolation is not the true value as the true u_{max} should be where the gradient for the slope is zero and in this case
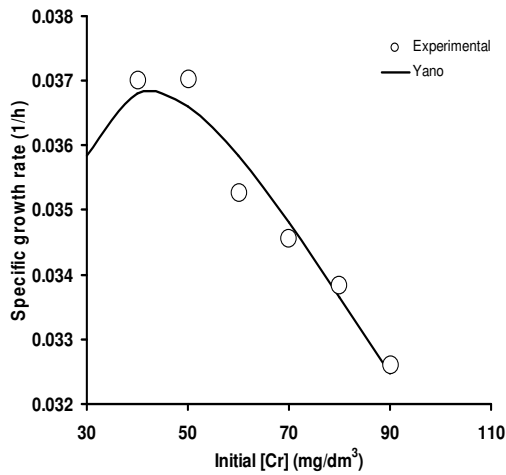
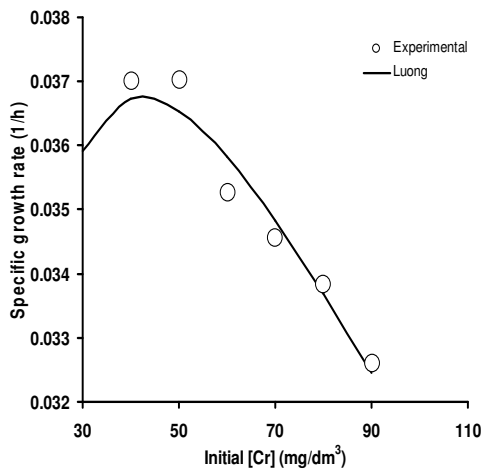Figure. 2. Fitting experimental data with the Yano model.

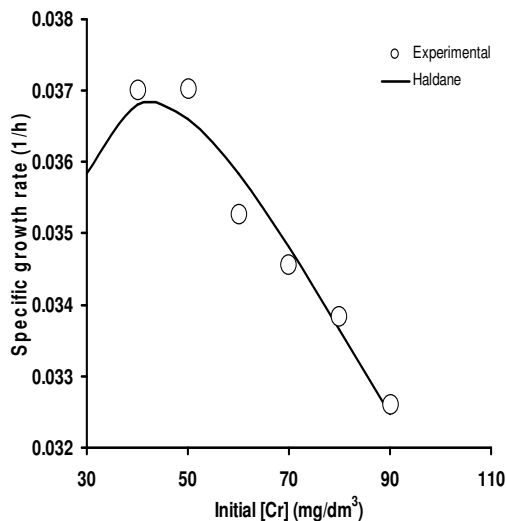Figure. 3. Fitting experimental data with the Luong model.

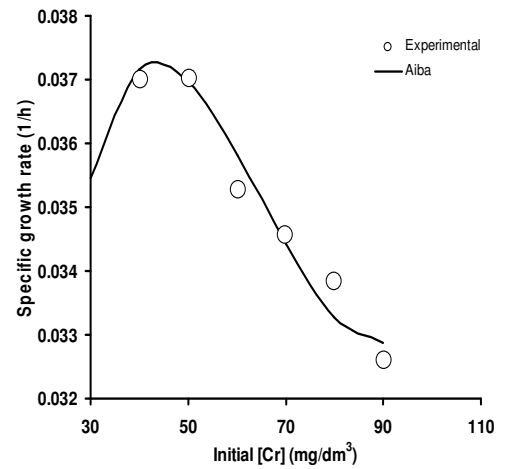Figure. 4. Fitting experimental data with the Haldane model.

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

(Haldane) the value was approximately 0.037 h^{-1} at 42 mg/dm^3 chromate (Figure 4).

Table 2. Statistical analysis of kinetic models.

Model	p	RMSE	R^2	adR^2	AiCC	BF	AF
Luong	4	0.0005	0.948	0.843	- 34.06	1.001	1.008
Aiba	4	0.0005	0.952	0.856	- 33.68	0.999	1.007
Yano	4	0.0005	0.956	0.867	- 35.02	1.001	1.007
Haldane	3	0.0004	0.956	0.911	- 79.02	1.001	1.007

note:

SSE	sums of squared errors
RMSE	root mean squared error
R^2	coefficient of determination
adR^2	adjusted coefficient of determination
AICC	corrected akaike information criterion
p	no of parameters
BF	bias factor
AF	accuracy factor

The most of the studies concerning substrate inhibition on microbial growth are carried out using toxic substrate such as aromatic and halogenated hydrocarbons [21,22] 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. From a biological perspective, xenobiotic such as chromate is toxic to biological system by virtue of its ability to inhibit enzymes and biological systems [23–26]. This indicates that the mathematical model developed based on enzyme inhibition such as Haldane and others do indeed have biological basis or mechanistic in properties and hence the parameters may have true biological meaning and not just empirical in character.

There were other models for describing substrate inhibition kinetics developed during this period such as the discontinuous models of Wayman and Tseng [27]. 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 [28]. 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 [4,5,29] 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.

Most studies on the reduction kinetics of heavy metals such as mercury [30], arsenate [31] and chromate [32] reported a Haldane-type inhibition by the substrate metal ions thus indicating the applicability and ubiquity of this model in fitting growth or biotransformation rate of heavy metals. Another model; the Luong model has been reported to optimally fit the molybdenum reduction rate in bacterium [29].

CONCLUSION

Both growth and degradation kinetics of bacteria can be modelled 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 modelling exercise was carried out on available published works to demonstrate this observation. In this work, we demonstrated based on statistical analysis that the Haldane model is the best model in fitting the growth kinetics data of *Bacillus* sp. (JUBTCr3) grown in the presence of chromate. We have added more statistical analysis to back up the published works.

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