

Evaluation of Several Mathematical Models for Fitting the Growth and Kinetics of the Catechol-degrading *Candida parapsilopsis*: Part 2

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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 remodelled the published work of Rigo et al. (2010) using several more growth kinetic models such as Monod, Haldane, 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 and Accuracy Factor. The accuracy and statistical analysis of the eleven kinetic models used shows that only the Haldane, Teissier, Moser, Aiba, Webb (Edward), Yano and Koga, and Luong could fit the data with the best model was Haldane with low values for RMSE and AICc, highest adjusted R^2 values, and with Bias Factor and Accuracy Factor nearest to unity (1.0). The calculated value for the Haldane constants maximal degradation rate, half saturation constant and half inhibition constant symbolized by μ_{max} , K_s and K_i , were 0.462 hr^{-1} , 112.21 mg/L and 211.34 mg/L , respectively. The true μ_{max} value occurs where the gradient for the slope is zero and in this case the value was approximately 0.188 h^{-1} at 155 mg/L catechol. 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

Catechol is a metabolic byproduct of phenol degradation by microbes. Its toxicity to water flea, trout, rabbit, cat, rat, zebra fish, mouse and for human cell lines has been long demonstrated [1–3]. Its presence in the environment at toxic concentrations has been demonstrated [4,5] and studied. For instance it reacts with sulphhydryl groups of proteins and glutathione leading to protein cross-linking and glutathione dimer formation and cause cessation of enzyme and metabolic activity in general [6]. Catechol in combination with heavy metals (e.g. Cu^{2+} , Fe^{3+}) and molecular oxygen causes DNA strand breaks [7,8]. It is also inhibitory to the oxidative phosphorylation in rat liver mitochondria [9]. Its degradation

and assimilation by microorganism is a potential tool for its bioremediation [10,11].

When a substrate inhibits its own biodegradation, the original Monod model becomes unsatisfactory. In this case, Monod derivatives that provided corrections for substrate inhibition (by incorporating the inhibition constant K_i) can be used to describe the growth-linked biodegradation kinetics [12]. A variety of microbial growth and biodegradation kinetic models have been developed, proposed and used by many researchers (Table 1). Such models allow prediction of chemicals that remain at a certain time, calculation of the time required to reduce chemical to certain concentration, estimation of how long it will take before a certain chemical concentration will be attained at a certain point (e.g. a case of aquifer, soil or

surface water) and design of bioremediation schemes in situ or ex situ to remove chemical contaminant to a designed concentration [13,14]. The generalization of the use of the Haldane model in literature to model substrate inhibition 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 [10,15,16]. Hence, the use of extensive models available could replace the Haldane in some circumstances. Without actually fitting these other models to the available growth rate data and proper statistical evaluation, the exclusive use of the Haldane model cannot be certified. Hence, the objective of this work is to evaluate similarities and differences between the models using published available data that lacks 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 graphs were scanned and electronically processed using WebPlotDigitizer 2.5 [17] which helps to digitize scanned plots into table of data with good enough precision [18,19]. Data were acquired from the works of Rigo et al.[11], from Figures 2 and 3 which show the effect of different concentration of the substrate catechol on the growth of *Candida parapsilopsis* measured over several hours and then replotted. The initial maximal growth rate or μ_m was obtained from fitting the growth curves using the Buchanan three phase model (Part 1 of this series).

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) and corrected AICc (Akaike Information Criterion) [20]. 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 [21].

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

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 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 [21].

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

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

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. 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 [22].

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

Author	Model	p	Author
Monod	$\mu_{max} \frac{S}{K_s + S}$	2	[23]
Haldane	$\mu_{max} \frac{S}{S + K_s + \frac{S^2}{K_i}}$	3	[24]
Teissier	$\mu_{max} \left(1 - \exp\left(-\frac{S}{K_i}\right) \right) \exp$	3	[25]
Andrews and Noack	$\mu_{max} \frac{S}{1 + \frac{K_s}{S} + \frac{S}{K_i}}$	3	[26]
Hinshelwood	$\mu_{max} \frac{S}{K_s + S} (1 - K_p P)$	4	[27]
Moser	$\mu_{max} \frac{S^n}{K_s + S^n}, n > 0$	3	[28]
Aiba	$\mu_{max} \frac{S}{K_s + S} \exp(-K_p)$	4	[29]
Webb (Edward)	$\mu_{max} S \left(1 + \frac{S}{K} \right) \frac{S^2}{S + K_s + \frac{S^2}{K_i}}$	4	[30]
Yano and Koga	$\frac{\mu_{max} S}{S + K_s + \left(\frac{S^2}{K_i} \right) \left(1 + \frac{S}{K} \right)}$	4	[31]
Han and Levenspiel	$\mu_{max} \left[1 - \left(\frac{S}{S_m} \right) \right]^n \frac{S}{S + K_s \left(1 - \left(\frac{S}{S_m} \right) \right)^n}$	5	[32]
Luong	$\mu_{max} \frac{S}{S + K_s} \left[1 - \left(\frac{S}{S_m} \right)^n \right]$	4	[16]

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 [21].

Accuracy Factor (AF) and Bias Factor (BF) to test for the goodness-of-fit of the models as suggested by Ross [33] were also used. The Bias Factor equal to 1 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 > 1 indicates a fail-safe model. The Accuracy Factor is always ≥ 1 , and higher AF values indicate less precise prediction.

$$\text{Bias factor} = 10^{\left(\sum_{i=1}^n \log \left(\frac{Pd_i / Ob_i}{n} \right) \right)} \quad (5)$$

$$\text{Accuracy factor} = 10^{\left(\sum_{i=1}^n \log \left(\left| \frac{Pd_i / Ob_i}{n} \right| \right) \right)} \quad (6)$$

RESULTS AND DISCUSSION

The results of the curve fitting are shown in **Figs. 1 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.

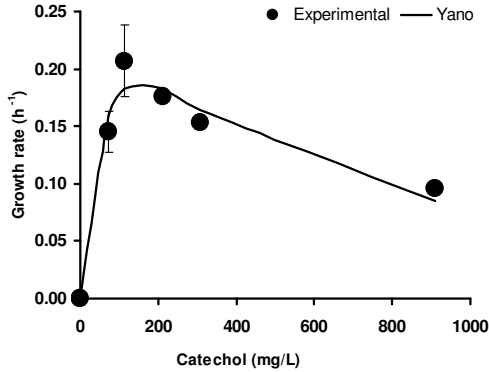


Fig. 1. Fitting experimental data with the Yano model.

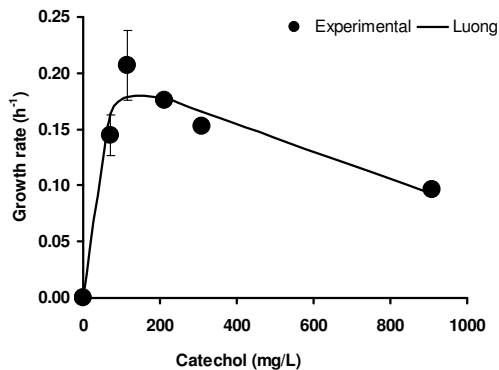


Fig. 2. Fitting experimental data with the Luong model.

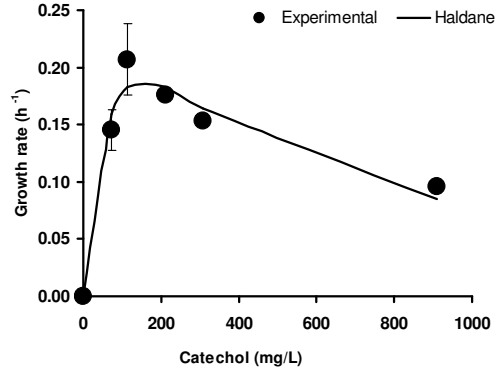


Fig. 3. Fitting experimental data with the Haldane model.

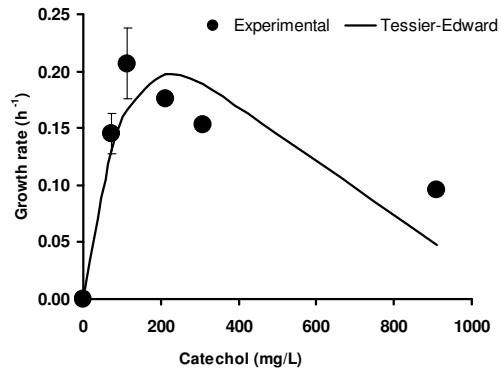


Fig. 4. Fitting experimental data with the Teissier-Edward model.

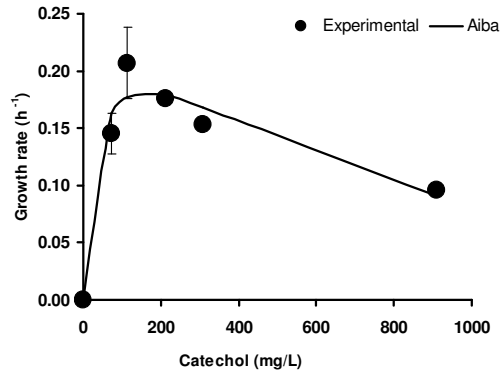


Fig. 5. Fitting experimental data with the Aiba model.

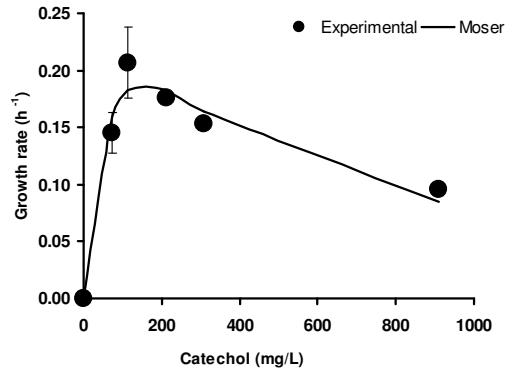


Fig. 6. Fitting experimental data with the Moser model.

Table 2. Statistical analysis of kinetic models.

Model	P	SSE	MSE	RMSE	R ²	adR ²	AICc	BF	AF
Luong	4	0.0014	0.0007	0.0260	0.947	0.733	18.13	1.004	1.072
Yano	4	0.0001	0.0001	0.0081	0.995	0.975	1.77	0.993	1.084
Tessier-Edward	3	0.0034	0.0011	0.0339	0.896	0.740	19.31	0.888	1.414
Aiba	4	0.0039	0.0019	0.0441	0.848	0.238	25.53	1.001	1.076
Haldane	3	0.0001	0.0000	0.0060	0.996	0.990	43.64	0.993	1.084
Webb	4	0.0002	0.0001	0.0101	0.992	0.961	4.88	1.007	1.048
Moser	3	0.0002	0.0001	0.0082	0.992	0.981	39.12	0.993	1.084

Note:

P	No of parameters
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
BF	Bias Factor
AF	Accuracy Factor

The accuracy and statistical analysis of the eleven kinetic models used shows that only the Haldane, Teissier, Moser, Aiba, Webb (Edward), Yano and Koga, and Luong could fit the data with the best model was Haldane with low values for RMSE and AICc, highest adjusted R^2 values, and with Bias Factor and Accuracy Factor nearest to unity (1.0) (Table 2). The calculated value for the Haldane constants maximal degradation rate, half saturation constant and half inhibition constant symbolized by μ_{max} , K_s and K_i , were 0.462 hr⁻¹, 112.21 mg/L and 211.34 mg/L, respectively. Rigo et al [11] modelled the growth rate kinetics using the Haldane model and obtained calculated values for the constants μ_{max} , K_s and K_i , at 0.246 hr⁻¹, 16.95 mg/L and 604.85 mg/L, respectively. The large difference in the values for the constants could be attributed to the low number of points available. A higher number of points would generate similar and more accurate values for the constants. It needs to be cautioned that the μ_{max} value obtained based on curve fitting interpolation is not the true value as the true μ_{max} should be where the gradient for the slope is zero and in this case the value was approximately 0.188 h⁻¹ at 155 mg/L catechol (Fig. 3).

Most of the studies concerning substrate inhibition on microbial growth are carried out using toxic substrate such as aromatic and halogenated hydrocarbons [34,35] 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. The Haldane model has been ubiquitously used as the general purpose model for describing substrate inhibition kinetics and has been used to model many bacterial growths on xenobiotics. The inhibition constant (K_i) indicates the concentration up to which cultures can tolerate shock loads. This value is particularly important for subsequent applications since it defines a concentration threshold that should not be exceeded.

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 was the best model in fitting the degradation kinetics data from the yeast strain grown on catechol from published literature. The assumption of the original author on

the applicability and reliability of the Haldane model was proven in this work using robust statistical analysis.

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