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Evaluation of Several Mathematical Models for Fitting the Growth and Kinetics of the Catechol-degrading *Candida parapsilopsis*: Part 1

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ABSTRACT

Predictive microbiology is a field often associated with food microbiology as it allows prediction of food spoilage. One of the key features of bacterial growth on solid and complex food under low water and oxygen is a long lag time. This long lag time is often observed in bacteria growing on toxic xenobiotics. In this work we modelled the growth of the yeast strain of *Candida parapsilopsis* based on available published work in the literature using several growth models such as modified logistic, modified Gompertz, modified Richards, modified Schnute, Baranyi-Roberts, Von Bertalanffy, Huang and the Buchanan three-phase linear model. Statistical analysis results indicated that the Buchanan model was the best with highest adjusted R^2, lowest RMSE and AICc values and Bias and Accuracy Factor values closest to unity. The fitted value of maximal growth rate showed a decline when the concentration of catechol was higher than 114 mg/L indicating substrate inhibition. The other fitted parameters such as lag period and maximal growth asymptote showed a general increase and a general decrease for the former and latter parameters, respectively. The results from this work can be used in the further optimization works of this alga in the future.

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]. The mechanism of toxicity has been studied. For instance it reacts with sulphydryl 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]. Like many xenobiotics, the growth on this toxic substrate exhibit a significant lag phase due to the needs of the cell to tolerate and initiate detoxification and degradation of enzymes upon exposure to catechol before assimilation can take place. The growth profile exhibits several phases where the specific growth rate starts at the value of zero followed by a stagnation of the rate associated with the lag time (λ). This is followed by acceleration to a maximal value (μ_m) for a given period of time. Finally the growth curves exhibit a final phase where the rate decreases and eventually reaches zero or an asymptote (A) [12]. A valuable parameter of the growth is the maximum growth rate (μ_m) [13]. This value is important for the development of secondary models such as growth kinetics [12]. In a large number of publications, this parameter is often estimated manually by deciding subjectively the part of the curve that is nearly linear and then the slope of this curve section is then determined usually by linear regression. A better method is to describe the entire set of data with a nonlinear regression growth model and then estimate μmax, λ, and A from the model. In addition many published works produced the growth curve but did not attempt any further to fitting the data to available models [12].

The sigmoidal curve can be fitted by different mathematical functions, such as Logistic [12,14], Gompertz [12,15], Richards [16], Schnute [12], Baranyi-Roberts [13] and Von Bertalanffy [17,18], Buchanan three-phase [19] and more recently Huang models [20] (**Table 1**). Apart from demonstrating predictive ability and internal consistency, which is a must, the usefulness of a model should also be judged by its mathematical simplicity, flexibility, the number of its adjustable parameters and, where appropriate, whether they have intuitive meaning.

The objective of the first part of this work is to evaluate similarities and differences between the models using published available data from Rigo et al [11] that lacks the initial modelling and to deal with the question of which model(s) can be used, on the basis of statistical reasoning. This would be followed by a second part where the evaluation of several growth kinetic models on the resultant initial growth rate against substrate concentration obtained from the first part would be carried out and compared to the model used by the same author but with further statistical analysis. This should give new data and results that could spur further information and improvement in the works already done by researchers.

Table 1. Growth models used in this study.

No	Model	No of parameters	Equation	Author
1	Modified Logistic	3	$y=\dfrac{A}{\left\{1+\exp\left[\dfrac{4\mu_{\max}}{A}(\lambda-t)+2\right]\right\}}$	(12)
2	Modified Gompertz	3	$y=A\exp\left\{-\exp\left[\dfrac{\mu_{\max}e}{A}(\lambda-t)+1\right]\right\}$	(12)
3	Modified Richards	4	$y=A\left\{1+v\exp(1+v)\exp\left[\dfrac{\mu_{\max}}{A}(1+v)\left(1+\dfrac{1}{v}\right)(\lambda-t)\right]\right\}^{\left(\frac{-1}{v}\right)}$	(12)
4	Modified Schnute	4	$y=\left(\mu_{\max}\dfrac{(1-\beta)}{\alpha}\right)\left[\dfrac{1-\beta\exp(\alpha\lambda+1-\beta-\alpha t)}{1-\beta}\right]^{\frac{1}{\beta}}$	(12)
5	Baranyi-Roberts	4	$y=A+\mu_{\max}x+\dfrac{1}{\mu_{\max}}\ln\left(e^{-\mu_{\max}x}+e^{-h_0}-e^{-\mu_{\max}x-h_o}\right)$ $-\ln\left(1+\dfrac{e^{\mu_{\max}x+\frac{1}{\mu_{\max}}\ln\left(e^{-\mu_{\max}x}+e^{-h_0}-e^{-\mu_{\max}x-h_0}\right)}-1}{e^{(y_{\max}-A)}}\right)$	(13)
6	Von Bertalanffy	3	$y=K\left[1-\left[1-\left(\dfrac{A}{K}\right)^3\right]\exp^{-\left(rx/3K^{\frac{1}{3}}\right)}\right]^3$	(17)
7	Huang	4	$y=A+y_{\max}-\ln\left(e^A+\left(e^{Y_{\max}}-e^A\right)e^{-\mu_{\max}B(x)}\right)$ $B(x)=x+\dfrac{1}{\alpha}\ln\dfrac{1+e^{-\alpha(x-\lambda)}}{1+e^{\alpha\lambda}}$	(20)
8	Buchanan Three-phase linear model	3	$y = A$, if $x < lag$ $y=A + k(x-\lambda)$, if $\lambda \leq x \geq x_{max}$ $y = y_{\max}$, if $x \geq x_{max}$	(21)

Note:
A= bacterial lower asymptote;
μ_{max}= maximum specific growth rate;
v= affects near which asymptote maximum growth occurs.
λ=lag time
y_{max}= bacterial upper asymptote;
e = exponent (2.718281828)
t = sampling time
α,β, k = curve fitting parameters
h_0 = a dimensionless parameter quantifying the initial physiological state of the cells. The lag time (day^{-1}) can be calculated as $h_0=\mu_{max}$

MATERIALS AND METHOD

Acquisition of Data

In order to process the data, the graphs were scanned and electronically processed using WebPlotDigitizer 2.5 [22] which helps to digitize scanned plots into table of data with good enough precision [23]. 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.

Fitting of the data

One of the growth data i.e. at catechol concentration of 308 mg/L was fitted nonlinearly using nonlinear regression software (CurveExpert Professional software, Version 1.6) that uses the Marquardt algorithm. This algorithm minimizes the sums of square of residuals between the predicted and experimental values. The program can be used in the manual mode or automatic mode where it calculates starting values by searching for the steepest ascent of the curve normally using four datum points to estimate the μ_{max}. The intersection of this line with the x axis is the estimation value of the lag time or λ while the final datum point is the estimation of 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.

Accuracy Factor (AF) and Bias Factor (BF) to test for the goodness-of-fit of the models as suggested by Ross [26] 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.

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

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

RESULTS AND DISCUSSION

One of the most important results from curve fitting in growth curve model is the ability to use a growth model that have a strong underlying mechanistic function based on sound theoretical knowledge of the system. One of the best of such model is the Michaelis-Menten kinetics that models the effect substrate on the initial enzyme activity of the enzyme. In order to find the best model, eight different growth models were used in this study to match the experimental data. The resultant fitting shows visually acceptable fitting for the models of Huang, modified Gompertz, Von Bertalanffy, Baranyi-Robert and Buchanan three phase models (**Fig. 1-4**). Other models gave poor fitting and were not shown. The statistical analysis results (**Table 2**) indicated that the Buchanan model was the best with highest adjusted R^2, lowest RMSE and AICc values and Bias and Accuracy Factor values closest to unity. The Buchanan three phase model was then used to fit the rest of the initial catechol concentrations and the resultant fitted values obtained (**Table 3**). The fitted value of maximal growth rate showed a decline when the concentration of catechol was higher than 114 mg/L indicating substrate inhibition. The other fitted parameters such as lag period and maximal growth asymptote showed a general increase and a general decrease for the former and latter parameters, respectively.

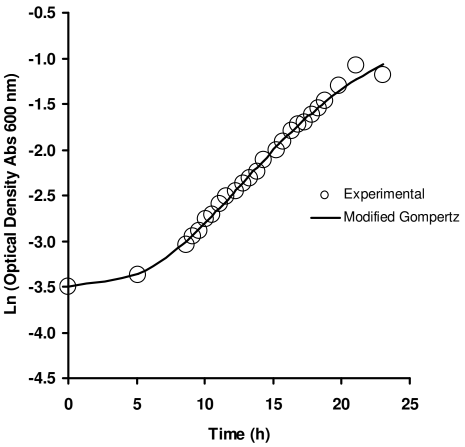

Fig. 1. Growth curves of *Candida parapsilopsis* fitted by the Modified Gompertz growth model. The optical density was transformed into natural logarithm.

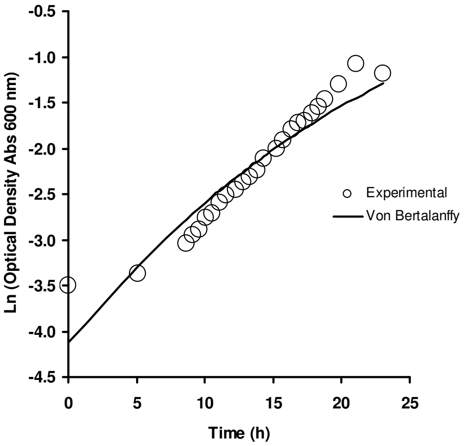

Fig. 2. Growth curves of *Candida parapsilopsis* fitted by the Von Bertalanffy growth model. The optical density was transformed into natural logarithm.

Table 2. Statistical analysis of the various fitting models.

Model	*p*	SSE	MSE	RMSE	R^2	adR^2	AICc	BF	AF
Modified Gompertz	3	0.0584	0.0027	0.0515	0.995	0.992	-142.67	1.001	1.008
Buchanan	3	0.0257	0.0012	0.0342	0.998	0.997	-163.20	1.000	1.007
Baranyi-Robert	4	0.2652	0.0126	0.1124	0.975	0.963	-102.60	1.002	1.027
Huang	4	0.0698	0.0033	0.0577	0.994	0.990	-135.96	1.000	1.009
Von Bertalanffy	3	1.0193	0.0463	0.2152	0.889	0.844	-71.19	1.018	1.043

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
BF	Bias Factor
AF	Accuracy Factor

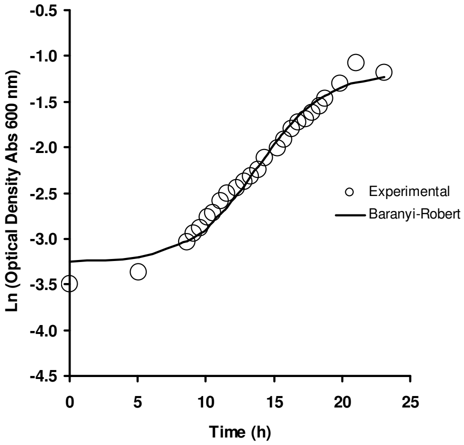

Fig. 3. Growth curves of *Candida parapsilopsis* fitted by the Baranyi-Robert growth model. The optical density was transformed into natural logarithm.

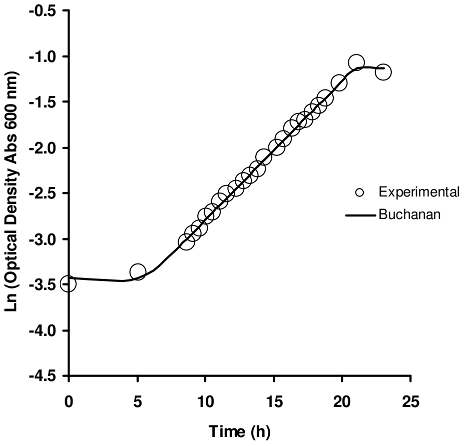

Fig. 4. Growth curves of *Candida parapsilopsis* fitted by the Buchanan three-phase growth model. The optical density was transformed into natural logarithm.

Table 3. Fitted growth parameters according to the Baranyi-Roberts model.

Catechol (mg/L)	λ	Lag Time (h)	y_0	y_{max}
72	0.145±0.018	2.927±0.855	-3.224±0.064	-1.867±0.045
114	0.207±0.031	5.759±0.730	-3.283±0.059	-1.928±0.021
213	0.176±0.005	6.576±0.299	-3.252±0.039	-1.404±0.021
308	0.153±0.002	5.814±0.207	-3.434±0.025	-1.130±0.025
910	0.096±0.001	4.799±0.476	-4.529±0.036	-1.351±0.027

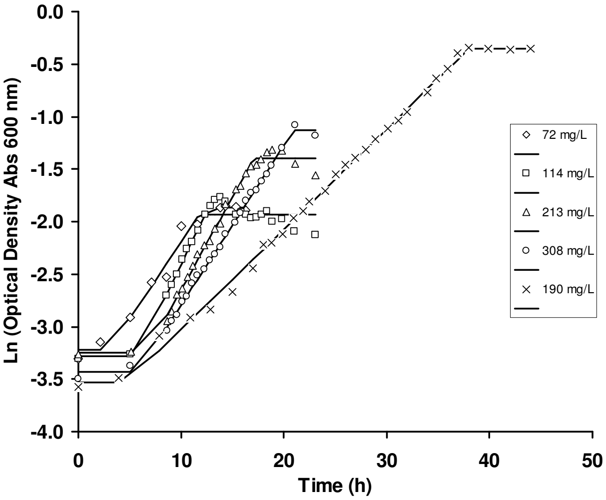

Fig. 5. Growth curves of *Candida parapsilopsis* at catechol concentrations of 72 (◇), 114 (□), 213 (△), 308 (○) and 910 (X) mg/L fitted by the Buchanan three-phase growth model. The optical density was transformed into natural logarithm.

Buchanan three phase growth model is one of the simplest mechanistic based predictive modeling to date. The traditional modeling practice hinges on the transformation of the observable exponential growth period of bacteria into a linear more manageable form in the form of natural logarithm (ln). Growth curve is then assigned to periods before (lag) and above (plateu) the exponential phase making the whole exercise a mechanistic in approach. The specific growth rate was then obtained via linear regression of the natural log-transformed portion of the exponential phase [19]. In the three-phase model, Buchanan limit the three phase into lines of constant concentration and abandon any attempt to model the smooth transitional curvature between phases, significantly simplifying the modeling process. Compare to the Gompertz equation, Buchanan three-phase model could model growth of bacteria under strong environmental pressure that results in long lag periods, minimal growth rate and the seemingly absence of stationary growth. Without an upper asymptote to model, the Gompertz model would run into difficulty whilst the solution adopts by the three-phase model is as simple as fixing N_{max} with value bigger than any of the observed concentrations [27]. The Buchanan three-phase models have been successfully used to model growth of bacteria [28–39], algae [40] and worm [41].

CONCLUSION

In conclusion, the various models used to fit the growth of *Candida parapsilopsis* on catechol as a substrate showed that the Huang, modified Gompertz, Von Bertalanffy, Baranyi-Robert and Buchanan three phase models could fit the experimental data but the best model according to statistical analysis is the Buchanan three phase. The results from this work can be used in the further optimization works of the algae.

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