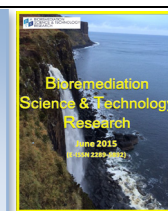




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Mathematical Modelling of the Growth of *Klebsiella pneumoniae* on 2-methylquinoline

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ABSTRACT

Quinolines compounds are toxic pollutants. Their biodegradation by microbes represents a tool for bioremediation. The growth of *Klebsiella pneumoniae* on 2-methylquinoline shows typical sigmoidal bacterial growth curves. Since there exists a variety of models for describing the growth profile of microorganism such as logistic, Gompertz, Richards, Schnute, Baranyi-Roberts, Von Bertalanffy, Buchanan three-phase and more recently Huang models, the growth curves exhibit under such conditions would be an excellent study for finding the best model. The Huang model was chosen as the best model based on statistical tests such as root-mean-square error (RMSE), adjusted coefficient of determination (R^2), bias factor (BF), accuracy factor (AF) and corrected AICc (Akaike Information Criterion). Novel constants obtained from the modelling exercise would be used for further secondary modelling.

INTRODUCTION

Quinolines exhibit carcinogenic and mutagenic properties. These compounds have been documented as toxic pollutants by the USEPA (United States Environmental Protection Agency) [1]. Quinolines are polar molecules with good water solubility. Hence, they are less retained by organic constituents in soils. These compounds are ubiquitously found in coal, creosote-based wood preservation products, oil shale, pharmaceuticals, pesticides and dyes [2]. The complete biodegradation of quinolines compounds by microbes is a potential tool for bioremediation. Several microbes have been isolated that could degrade quinolines with a majority of them form the metabolite 8-hydroxycoumarin as an important intermediate. These microbes are generally bacteria and include *Pseudomonas* sp., *Acinetobacter* strain from oil and creosote contaminated soils, *Pseudomonas aeruginosa*, *Pseudomonas putida* and *Pseudomonas ayucida* IGTN9m [1-7].

Primary modelling is hardly ever employed in bacterial growth on xenobiotics or detoxification processes because the latter is often times utilized in food microbiology modelling. Models for example modified Gompertz, modified logistics, Huang,

Buchanan-three phase and Baranyi and Roberts are definitely the one of the most popular as they are able to precisely model the growth of bacteria [8]. In the secondary modelling exercises, the consequence of substrate or environmental factors on the growth or bacteria metabolic rates is studied. Almost all models that are widely-used in modelling bacterial growth or metabolite production rates are regarded as either empirical or mechanistic, even though the truth is many of them lie in between those two categories [9]. Bacterial growth on substrates including quinolines often exhibit a distinctive phase when the specific growth rate begins with a value of zero and after that speeds up to a maximum value (μ_{max}) in a given time period, resulting in a lag time (λ). In the final phase, the rate diminishes and eventually becomes zero, reaching an asymptote (A). The overall profile is a sigmoidal curve [10]. Another valuable parameter of the growth curve aside from the asymptotic value and the lag period is the maximum specific growth rate (μ_m). This value is often used in the development of secondary models such as the effects of substrate, product, pH and temperature on growth rate of microorganism. Furthermore, the lag phase is often discernably seen to increase as

the concentration of toxic substrate is utilized. This sigmoidal curve can be fitted by different mathematical functions, including the modified Gompertz [11,12], Schnute [12,13], Richards [12,14], the Logistic [12,15], Baranyi-Roberts [16] Von Bertalanffy [17], Buchanan three-phase [18] and more recently Huang models [8]. Thus, the goal of this work is to assess the ideal model according to current mathematical models for bacterial growth and backed up by statistical thinking. This absolutely should provide new data and outcomes that may encourage more information and enhancement in the works already carried out.

METHODOLOGY

Acquisition of Data

Data were acquired from the works of Wang et al. [19] from Figure 3(b) which shows the effect of different concentrations of 2-methylquinoline on the growth of *Klebsiella penumoniaee* TJ-A measured at OD 600 nm (Fig. 1). Data from the figure was scanned and electronically processed using the software WebPlotDigitizer 2.5 [20]. The program digitally scanned plots into data with good enough precision [21,22].

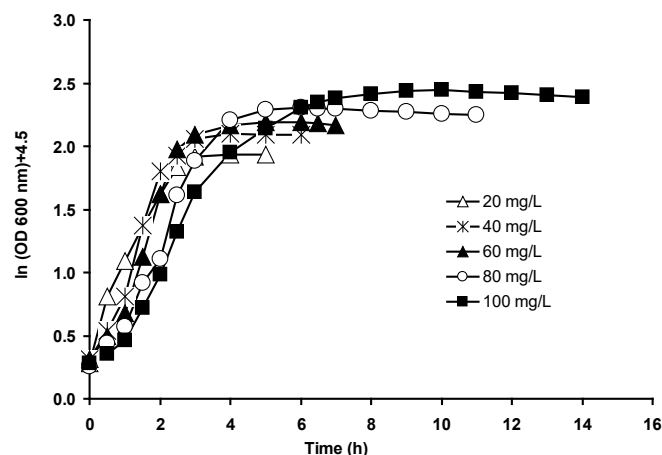


Fig. 1. Replotted growth curves *Klebsiella penumoniaee* TJ-A. The optical density was transformed into natural logarithm.

Fitting of the data

Fitting of the growth data to various growth equations (Table 1) were done by nonlinear regression using the Marquardt algorithm. This algorithm minimizes the sums of square of residuals. In this lookup approach the sum of the squares of the differences between the predicted and observed values is minimized. The program utilized was the CurveExpert Professional software (Version 1.6). The software can be automatically or manually programmed to calculate initial values of parameters. Estimation of μ_m was carried out by the steepest ascent search of the curve amongst four datum points. The Runge-Kutta method was utilized to solve numerically differential equation in some of the models. The ode45 solver in MATLAB (Version 7.10.0499, The MathWorks, Inc., Natick, MA) was used to solve this equation.

Statistical analysis

To make a decision whether there's a statistically significant difference between models with different number of parameters, with regards to the quality of fit to the same experimental data was statistically evaluated through numerous methods including the corrected AICc (Akaike Information Criterion), Root-Mean-Square Error (RMSE), bias factor (BF), accuracy factor (AF), and adjusted coefficient of determination (R^2) [23–25].

Table 1. Growth models used in this study.

Model	n	Equation
Modified Logistic	3	$y = \frac{A}{1 + \exp\left[\frac{4\mu_{\max}}{A}(\lambda - t) + 2\right]}$
Modified Gompertz	3	$y = A \exp\left\{-\exp\left[\frac{\mu_{\max}}{A}(\lambda - t) + 1\right]\right\}$
Modified Richards	4	$y = A \left\{1 + v \exp(1 + v) \exp\left[\frac{\mu_{\max}}{A}(1 + v)\left(1 + \frac{1}{v}\right)(\lambda - t)\right]\right\}^{\left(\frac{-1}{v}\right)}$
Modified Schnute	4	$y = \left(\mu_{\max} \frac{(1 - \beta)}{\alpha}\right) \left[\frac{1 - \beta \exp(\alpha\lambda + 1 - \beta - \alpha t)}{1 - \beta}\right]^{\frac{1}{\beta}}$
Baranyi-Roberts	4	$y = A + \mu_{\max} x + \frac{1}{\mu_{\max}} \ln\left(\frac{e^{-\mu_{\max}x} + e^{-h_0} - e^{-\mu_{\max}x - h_0}}{\mu_{\max}}\right) - \ln\left(1 + \frac{e^{\frac{\mu_{\max}x + 1}{\mu_{\max}} \ln(e^{-\mu_{\max}x} + e^{-h_0} - e^{-\mu_{\max}x - h_0})} - 1}{e^{(y_{\max} - A)}}\right)$
Von Bertalanffy	3	$y = K \left[1 - \left[1 - \left(\frac{A}{K}\right)^3\right] \exp\left\{-\left(\frac{\mu_{\max} x}{3K}\right)^3\right\}\right]^{\frac{1}{3}}$
Huang	4	$y = A + y_{\max} - \ln\left(e^A + (e^{y_{\max}} - e^A)e^{-\mu_{\max}B(x)}\right)$ $B(x) = x + \frac{1}{\alpha} \ln \frac{1 + e^{-\alpha(x-\lambda)}}{1 + e^{\alpha\lambda}}$
Buchanan Three-phase linear model	3	$Y = A, \text{ IF } X < \text{LAG}$ $Y = A + K(X - \lambda), \text{ IF } \lambda \leq X \leq X_{MAX}$ $Y = Y_{MAX}, \text{ IF } X > X_{MAX}$

Note:

n= no of paramaters

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 $h_0 = \mu_{\max}$

The coefficient of determination or R^2 is used to assess the quality of fit of a model in linear regression. However, in nonlinear regression where difference in the number of parameters between one models to another do vary, the adoption of the R^2 method does not readily provides comparable analysis. In order to solve this issue the adjusted R^2 is used to calculate the quality of nonlinear models using equations i and ii according to the formula where RMS is the Residual Mean Square and the total variance of the y-variable is represented by s_y^2 .

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

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

Accuracy Factor (AF) and Bias Factor (BF) (Eqns. iii and iv) to test for the goodness-of-fit of the models was calculated according to Eqns. iii and iv as suggested and first proposed by Ross [26]. A Bias Factor that is equal to 1 signifies an ideal match between observed and predicted values. For microbial growth curves, a bias factor having values < 1 signifies a fail-dangerous model whilst a bias factor having values > 1 signifies a model that is fail-safe. The value of the Accuracy Factor is usually ≥ 1 , with higher AF values signifies prediction that is less precise or accurate.

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

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

RESULTS AND DISCUSSION

Eight different growth models (Table 1) were used in this study to match the experimental data. The resultant fitting shows visually acceptable fitting (Fig. 2) with the exception of the modified Shnute and Baranyi-Roberts model. The Huang model incidentally gave the most significant results as the most accurate model based on almost all statistical tests and was utilized to model the growth data (Fig. 3). The poorest performance was modified Schnute with the lowest score for all statistical tests (Table 2).

Parameters obtained from the fitting exercise showed an approximate lag period increase as the substrate was increased. In addition, the growth rate exhibited substrate inhibition at substrate concentrations higher than 40 mg/L (Table 3). These biologically meaningful coefficients would be later used for secondary modelling of Mo-blue production using model such as the two-parameter Monod model or other more complex models "secondary models" such as Haldane, Aiba, Yano and others. These mechanistic models are used in basic research and are aimed to reach a better understanding of the physical, chemical and biological processes that lead to the growth profile seen. All other things being equal, mechanistic models are more powerful since they tell you about the underlying processes driving patterns. They are more likely to work correctly when extrapolating beyond the observed conditions [27].

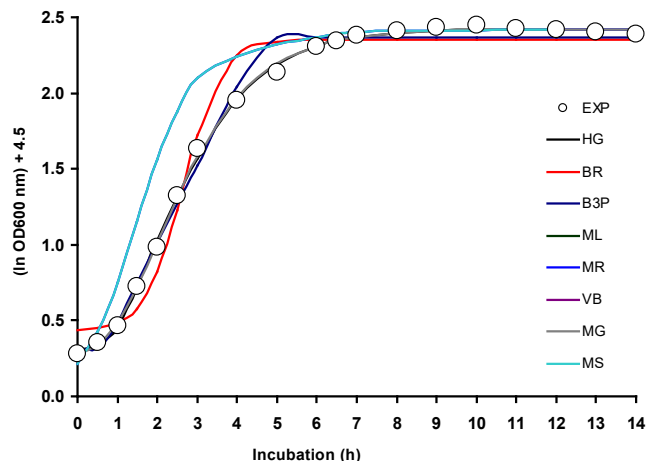


Fig. 2. Growth curves of *Klebsiella pneumoniae* TJ-A fitted by various growth models. The optical density was transformed into natural logarithm.

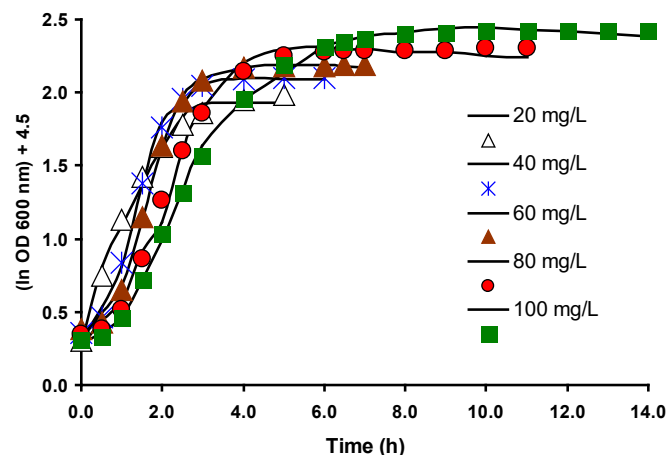


Fig. 3. Growth curves of *Klebsiella pneumoniae* TJ-A fitted by the Huang model. The optical density was transformed into natural logarithm.

Table 2. Statistical analysis of the various fitting models.

Model	n	RMSE	R^2	adR^2	AF	BF	AICc
modified Schnute	4	0.33	0.83	0.78	1.16	1.12	-24.48
modified Richards	4	0.29	0.87	0.83	1.16	1.12	-28.91
modified Logistics	3	0.28	0.87	0.84	1.16	1.12	-34.67
von Bertalanffy	3	0.28	0.87	0.85	1.22	1.06	-35.35
Baranyi-Roberts	4	0.13	0.98	0.97	1.10	1.02	-58.75
modified Gompertz	3	0.12	0.98	0.97	1.04	1.00	-66.19
Buchanan-3-phase	3	0.08	0.99	0.99	1.04	1.00	-81.88
Huang	4	0.03	1.00	1.00	1.02	1.00	-117.08

Note:

p no of parameters
 adR^2 Adjusted Coefficient of determination
 BF Bias factor
 AF Accuracy factor

Table 3. Fitted growth parameters according to the Huang model.

Parameters	2-methylquinoline concentration (mg/L)				
	20	40	60	80	100
Y_0 (ln OD600 nm)	0.299	0.358	0.38	0.349	0.306
Lag (h)	-4.834	0.756	0.991	1.013	0.917
Y_{max} (ln OD600 nm)	1.989	2.098	2.185	2.295	2.421
μ_{max} (h ⁻¹)	1.17	2.003	1.932	1.207	0.817

In the model proposed by Huang [8,28], the first consideration is to describe the growth of a bacteria or increased in a growth – associated product with a 1st-order growth kinetics that does not take into account the lag and stationary phase yet. The model identifies the duration of the lag phase (λ) rather clearly. The variables in the equation such as y_0 , y_{max} , and $y(t)$, are identical to the parameters in the Baranyi-Roberts model. The transition coefficient (α) is in fact not easy to be determined from the growth curve by nonlinear regression especially so when data points from the lag phase is not enough. This coefficient specifies the transition from the lag phase towards the exponential phase. As a result, the transition coefficient was suggested arbitrarily to be large enough (i.e. 25). The selection of α value as 25 allows the quick and smooth transition of the lag to the exponential phase of the growth curve. In addition, this allow the lag phase to be determined accurately. Furthermore convergence of nonlinear regression using a few data points is also quick at this value.

CONCLUSION

In conclusion, The Huang model was the best model in modelling the growth curves of *Klebsiella pneumoniae* TJ-A on 2-methylquinoline based on statistical tests such as root-mean-square error (RMSE), adjusted coefficient of determination (R^2), bias factor (BF), accuracy factor (AF) and corrected AICc (Akaike Information Criterion). Current works include secondary modelling of the growth constants from the Huang model especially on the inhibitory effect of the substrate on growth rate.

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