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Research article

Modeling the growth curves of *Acinetobacter* sp. strain DRY12 grown on diesel

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ABSTRACT

Diesel is a toxic xenobiotic. However, some bacteria are capable of using diesel as a carbon source for energy and growth. At high diesel concentration, an inhibition to the growth curves is seen. The study of the growth inhibitory properties of diesel on bacterium has often been carried out through the modelling of the specific growth rates obtained from the linearization of the growth curves of bacterium. Since there exists a variety of models for obtaining a more accurate specific growth rates from bacterial growth curves such as logistic, Gompertz, Richards, Schnute, Baranyi-Roberts, Von Bertalanffy, Buchanan three-phase and more recently Huang models, the use of these models over the simplification through linearization is needed. The modified Gompertz model gave the best fitting based on statistical test with the lowest values for RMSE and corrected Akaike Information Criteria, the highest value for adjusted R² and the closest values to unity for both Accuracy and Bias factors. The modified Gompertz model is thus the best model to obtain specific growth rates from the growth curves of *Acinetobacter* sp. strain DRY12 grown on diesel.

INTRODUCTION

Bacterial growth often shows a phase in which the specific growth rate starts at a value of zero and then accelerates to a maximal value (μ_{max}) in a certain period of time, resulting in a lag time (λ). In addition, growth curves contain a final phase in which the rate decreases and finally reaches zero, so that an asymptote (a) is reached. usually these growth rate changes result in a sigmoidal curve, with a lag phase just after $t = 0$ followed by an exponential phase and then by a stationary phase [1]. Besides the lag period and the asymptotic value, another valuable parameter of the growth curve is the maximum specific growth rate (μ_{max}). This value is often used in the development of secondary models that model the effect of environmental conditions such as pH, water activity and temperature on growth rate of organism. since the logarithm of the number is used, μ_{max} is given by the slope of the line when the organisms grow exponentially [2]. Usually this parameter is estimated by deciding subjectively which part of the curve is approximately linear and then determining the slope of this curve section, eventually by linear regression. Some authors indeed use growth models to describe their data but resort to linearize the sigmoidal curve by logarithmic data transformation. a better method is to describe the entire set of data with a nonlinear regression growth model and then estimate μ_{max} , λ , and a from the model [3]. In addition many published works produced the growth curve but did not attempt any further to fitting the data to available models.

Previously, a diesel-degrading bacterium has been isolated from soils and the kinetics of the growth of the bacterium determined using various inhibitory growth kinetics models [4]. In order to obtain the specific growth rate needed to model the kinetics, linearization of the non-linear growth curves is carried out.

Hence, the objective of this work is to evaluate similarities and differences between available models such as logistic [1,5], Gompertz [1,6], Richards [1,7], Schnute [1], Baranyi-Roberts [8], Von Bertalanffy [9,10], Buchanan Three-Phase [11] and more recently Huang model [12] (table 1) in modeling the growth curves of the bacterium *Acinetobacter* sp. strain DRY12 grown on diesel as a carbon source. This should give a better data for the specific growth rates to be used in secondary modeling studies.

MATERIALS AND METHODS

Growth and maintenance of *Acinetobacter* sp. strain DRY12

Acinetobacter sp. strain DRY12 from (4) was grown in a 100 ml media consisted of a modified basalt salt media at pH 7.0 (supplemented with 1% (v/v) diesel as carbon source) composed of (per liter of distilled water): KH_2PO_4 , 1.360 g; Na_2HPO_4 , 1.388 g; MgSO_4 , 0.01 g; CaCl_2 , 0.01 g; $(\text{NH}_4)_2\text{SO}_4$, 7.7 g; and 100 ml of a mineral solution containing 0.01 g of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, H_3BO_3 , $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{Fe}_2\text{SO}_4 \cdot 2\text{H}_2\text{O}$, $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$ [13]. The flasks were incubated at 30 °C and 150 rpm (YIH DER, Taiwan) for six days. For growth studies, the medium was supplemented with various concentrations of diesel and incubated for 10 days. Bacterial growth was measured as colony-forming-unit or CFU/ml.

Table 1. Growth models used in this study.

No	Model	p	Equation
1	Modified Logistic	3	$y = \frac{A}{1 + \exp\left[\frac{4\mu_{max}}{A}(\lambda - t) + 2\right]}$
2	Modified Gompertz	3	$y = A \exp\left\{-\exp\left[\frac{\mu_{max} e}{A}(\lambda - t) + 1\right]\right\}$
3	Modified Richards	4	$y = A \left\{1 + v \exp(1 + v) \exp\left[\frac{\mu_{max}}{A}(1 + v) \left(1 + \frac{1}{v}\right)(\lambda - t)\right]\right\}^{\left(\frac{-1}{v}\right)}$
4	Modified Schnute	4	$y = \left(\mu_{max} \frac{(1 - \beta)}{\alpha}\right) \left[\frac{1 - \beta \exp(\alpha\lambda + 1 - \beta - \alpha t)}{1 - \beta}\right]^{\frac{1}{\beta}}$
5	Baranyi-Roberts	4	$y = A + \mu_{max} x + \frac{1}{\mu_{max}} \ln\left(e^{-\mu_{max} x} + e^{-h_0} - e^{-\mu_{max} x - h_0}\right) - \ln\left(1 + \frac{e^{\frac{\mu_{max} x + \frac{1}{\mu_{max}} \ln(e^{-\mu_{max} x} + e^{-h_0} - e^{-\mu_{max} x - h_0})}{e^{(y_{max} - A)}}} - 1}{e^{(y_{max} - A)}}}\right)$
6	Von Bertalanffy	3	$y = K \left[1 - \left[1 - \left(\frac{A}{K}\right)^3\right] \exp\left\{-\left(\mu_{max} x / 3K\right)^{\frac{1}{3}}\right\}\right]^3$
7	huang	4	$y = A + y_{max} - \ln\left(e^A + \left(e^{y_{max}} - e^A\right)e^{-\mu_{max} B(x)}\right)$ $B(x) = x + \frac{1}{\alpha} \ln \frac{1 + e^{-\alpha(x-\lambda)}}{1 + e^{\alpha\lambda}}$
8	buchanan three-phase linear model	3	$y = a, \text{ if } x < \lambda$ $y = a + k(x - \lambda), \text{ if } \lambda \leq x \leq x_{max}$ $y = y_{max}, \text{ if } x \geq x_{max}$

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

p =no of parameters

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 $h_0 = \mu_{max}$

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).

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 [14].

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

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.

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

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

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 [15]. 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 [14].

Accuracy factor (AF) and bias factor (BF) to test for the goodness-of-fit of the models as suggested by Ross [16] 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.

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

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

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). Of all the models tested (Table 1) indicate mixed results.

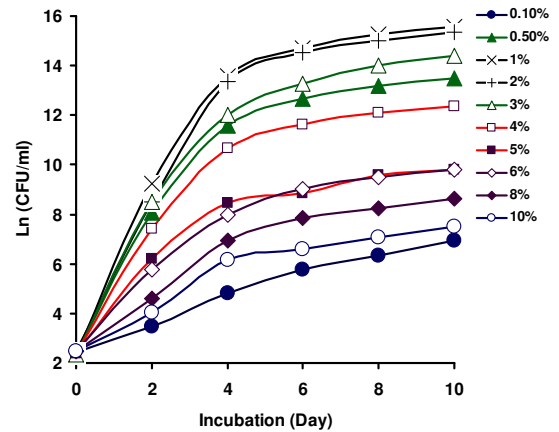


Figure 1. The growth of curves of *Acinetobacter* sp. strain DRY12 on various concentrations of diesel. The coefficient of variation for all data was less than 10% for all data and error bars were omitted for clarity.

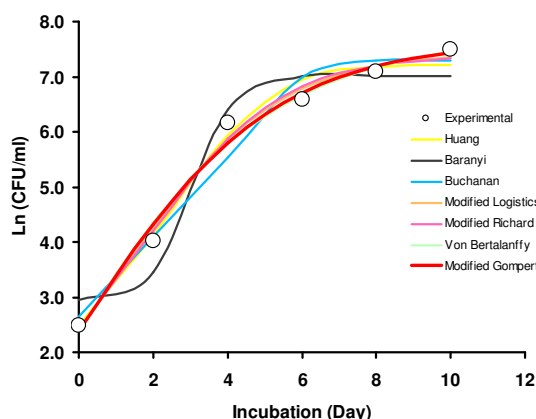


Figure 2. The growth of curve of *Acinetobacter* sp. strain DRY12 at 0.1% (v/v) diesel fitted by various growth models available in the literature. The coefficient of variation for all data was less than 10% for all data and error bars were omitted for clarity.

Table 1. Statistical analysis of the various fitting models.

Model	<i>p</i>	RMSE	R ²	adR ²	AICc	BF	AF
Huang	4	0.3691	0.986	0.928	55.28	1.001	1.028
Baranyi-robert	4	0.7104	0.945	0.724	64.44	1.007	1.087
Buchanan	3	0.4716	0.964	0.910	17.55	1.005	1.052
Modified logistics	3	0.2695	0.989	0.972	9.71	0.999	1.034
Modified richard	4	0.3269	0.989	0.944	53.58	0.999	1.032
Von bertalanffy	3	0.3079	0.985	0.963	11.58	0.999	1.037
Modified Gompertz	3	0.2460	0.991	0.976	8.44	0.999	1.036

note:

p no of parameters
 Ra² adjusted coefficient of determination
 BF bias factor
 AF accuracy factor

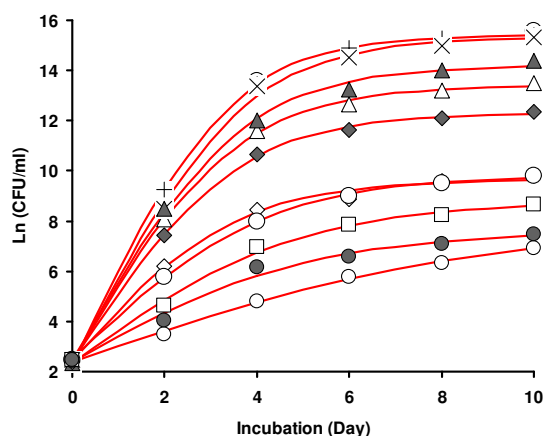


Figure 3. The growth of curves of *Acinetobacter* sp. strain DRY12 on various concentrations of diesel fitted using the modified Gompertz model. The coefficient of variation for all data was less than 10% for all data and error bars were omitted for clarity.

The modified Gompertz model gave the best fitting based on statistical test with the lowest values for RMSE and corrected Akaike Information Criteria, the highest value for adjusted R² and the closest values to unity for both accuracy and bias factors. The poorest performance was Baranyi-Robert with the lowest score for all statistical tests. The modified Gompertz model is one of the classical growth models that include model such as the Verhulst [1,6]. The Gompertz function, named in 1844-1845 by Pierre François Verhulstis, is based on an exponential relationship between specific growth rate and population density. The initial stage of growth is approximately exponential; then, as saturation begins, the growth slows, and at maturity, growth stops. the modified function is suited for microbial growth and has incorporated constant such as the lag period [1]. It is a three-parameter model. In general, without resorting to complicated statistical treatment, a three-parameter model is recommended over a four-parameter if it is sufficient to describe the data. This is because the three-parameter model is simpler, easier to use, the solution is more stable, the estimates have more degrees of freedom and all three parameters can be given a biological meaning. In addition, the fourth or fifth parameter in the four- or five-parameter models functions mostly as shape parameter and usually has no biological interpretation. these models can be found useful and more accurate if a large number of datum points are available [10].

The modified Gompertz model has been extensively used to model the growth of bacteria with more than 50% of all growth models studies citing the use of the model. examples of its usage for modelling the growth curves of bacteria are abundant [17–28] in the literature.

CONCLUSION

In conclusion the modified Gompertz model was the best model in modeling the growth curves of the bacterium *Acinetobacter* sp. strain DRY12 grown on diesel as a carbon source. The use of bacterial growth models to obtained growth rates data for secondary model development is very scarce in the literature and this work has demonstrated the applicability of such models. Current works include the modeling of the growth kinetics of this bacterium using the specific growth rates data obtained from this works.

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