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Mathematical Modelling of the Growth Curve of *Vibrio* sp. Isolate MZ Grown in Seawater Medium

Maryam Zahaba[1,2], Mohd. Izuan Effendi Halmi[3], Ahmad Razi Othman[4], Mohd. Yunus Shukor[1]*

[1]Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, UPM 43400 Serdang, Selangor, Malaysia.
[2]Department of Chemistry, International Islamic University Malaysia (IIUM), Jalan Sultan Haji Ahmad Shah, Indera Mahkota, 25200, Kuantan, Pahang Malaysia.
[3]Department of Land Management, Faculty of Agriculture, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
[4]Research Centre for Sustainable Process Technology (CESPRO), Faculty of Engineering and Built Environment, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia

*Corresponding author:
Mohd. Yunus Shukor
Department of Biochemistry,
Faculty of Biotechnology and Biomolecular Sciences,
Universiti Putra Malaysia,
UPM 43400 Serdang,
Selangor,
Malaysia.
Email: mohdyunus@um.edu.my / yunus.upm@gmail.com

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ABSTRACT

Pollution in the environment is deteriorating the ecology due to human activities in a large array of industrial and agricultural sectors. Bioassay of polluted waters using bioluminescent bacterium has been touted as one of the most economical, rapid and sensitive tests. The growth of the bacterium on seawater medium exhibited a typical sigmoidal profile. To extract important growth parameters useful for further modelling exercise, various primary growth models were utilized in this study such as Modified Logistic, modified Gompertz, modified Richards, modified Schnute, Baranyi-Roberts, von Bertalanffy, Huang and the Buchanan three-phase model. The best performance was Huang model with the lowest value for RMSE, AICc and the highest value for adjusted R^2. The AF and BF values were also excellent for the model with their values were the closest to 1.0. The Huang parameters, which include A or Y_0 (bacterial growth lower asymptote), μ_m (maximum specific bacterial growth rate), λ (lag time) and Y_{max} (bacterial growth upper asymptote) were 7.866 (95% confidence interval of 7.850 to 7.883), 0.329 (95% confidence interval of 0.299 to 0.359), 1.543 (95% confidence interval of 1.303 to 1.784) and 8.511 (95% confidence interval of 0.299 to 0.359).

INTRODUCTION

A lot of chemical contaminants, which may possess potential toxicity and carcinogenicity, are released to the environment [1]. These compounds mainly sink into soil and groundwater. In some cases, especially for complex contamination, the sunken compounds alter soil properties. Therefore, microbiological decontamination or bioremediation is claimed to be an efficient, economic, and adaptable alternative [2]. As reported by [3], *Rhodococcus* bacteria are considered to be promising degraders of persistent pollutants and can be utilised in biological preparations for contaminated wastewater and soil cleanup. Nowadays, luminescence-based system is available. However, the systems such as Microtox and ToxAlert that use *Vibrio fisheri* NRRL B-111 77 are not ideal because they require exact 15 °C. Hence, the cost of using the system will be increased due to usage of thermostat. In our previous work, we develop a tropical-climate based luminescence bacterial system using the bacterium *Vibrio* sp. isolate MZ to overcome this limitation and to evaluate toxicity profile during bioremediation process of hydrocarbon. To obtain reproducible growth data of the bacterium for routine harvesting of the bacterium for monitoring purpose, it is often that the growth of the bacterium is monitored. However, to date, primary modelling of the growth of luminescent bacterium is lacking.

Normally, bacterial growth curve exhibited a sigmoidal pattern, beginning with the lag phase just after t =0, followed by

the exponential phase and then the bacteria enters the stationary phase and eventually a decline in growth or the death phase [4]. Valuable parameters of the growth curve are the asymptotic values, the lag period and the maximum specific growth rate (μ_m). The latter value can be used in the development of secondary models to study the effects of substrate, product, pH and temperature on growth rate.

Most bacterial growth models lie between an empirical and mechanistic properties, although it is likely that these two classifications exist in reality side by side [4]. In this work the use of primary models in modelling the growth curve of *Vibrio* sp. isolate MZ is presented for the first time.

MATERIALS AND METHODS

Maintenance of luminescence bacterium

Vibrio sp. isolate MZ was maintained in seawater (SW) medium prepared and modified according to [5]. The composition of the medium was as follows: 1% sodium chloride (w/v), 10 g/L peptone, 10 g/L sucrose, 0.5 g/L yeast extract and 2 g/L calcium carbonate. The final pH of the medium was adjusted to 8.4 using 50 mM Tris-HCl buffer. To solidify the medium, 18 g/l of bacteriological agar was added. Maintenance of luminescence bacterium was done on slanted SW agar in universal bottles. The universal bottles were tightly capped and kept at 4 °C in the refrigerator as stock culture.

The storage duration for the slant stock cultures was between one to three months. The second method for maintenance of luminescence bacterium was using Microbank™. Other than these methods, the pure culture grown in SW medium was preserved at −20 °C in Eppendorf tubes containing 20% (v/v) glycerol [6]. 10% of bacterial culture (OD_{620} = 0.8-1.0) was inoculated into 50 mL of SW media and grown on shaker at 150 rpm. OD600 nm values were converted to CFU/mL using the formula of OD600nm value of 1.0 is equal to 2.3 x 10^8 CFU/mL. The experiment was carried out at room temperature and pH 8.4.

Statistical analysis

Statistical significant difference between the models was 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) as before [7].

Fitting of the data

Fitting of the bacterial growth curve using various growth models (**Table 1**) was carried out using the CurveExpert Professional software (Version 1.6) by nonlinear regression utilizing the Marquardt algorithm. Estimation of μ_m was carried out by the steepest ascent search of the curve while the intersection of this line with the x axis is an estimation of λ.

Finally, the final datum point is an estimation for the asymptote (A). The Huang's model needs to be solved numerically as it is differential equation. The Runge-Kutta method through the ode45 solver in MATLAB (Version 7.10.0499, The MathWorks, Inc., Natick, MA) was utlized.

Table 1. Growth models used in this study.

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

Note:
A= Bacterial growth lower asymptote;
μ_m= maximum specific bacterial growth rate;
v= affects near which asymptote maximum growth occurs.
λ=lag time
y_{max}= Bacterial growth 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 reduction process. The lag time (h^{-1}) can be calculated as $h_0=\mu_{max}$

RESULTS AND DISCUSSION

The Huang model is one of theoretical models developed in recent years [7]. The model clearly defines the duration of the lag phase and is based on the fundamental growth phenomenon of microorganism. The model also defined the exponential growth rate clearly. Since then, the model has been successfully utilized to model numerous microorganisms' growth [8–18] indicating the utility of the model. The bacterial growth curve was sigmoidal in shape with a lag phase of about 1 h and reaching maximum growth at approximately after 20 h of incubation (**Fig 1**). The bacterial growth curve over time profile was fitted to eight different models. The resultant fitting shows visually acceptable fitting (**Figs. 2 to 7**).

The best performance was Huang model with the lowest value for RMSE, AICc and the highest value for adjusted R^2. The AF and BF values were also excellent for the model with their values were the closest to 1.0. The poorest performance was modified Schnute where it failed to model the growth curve (**Table 2**). The coefficients for the Huang model is shown in **Table 3**.

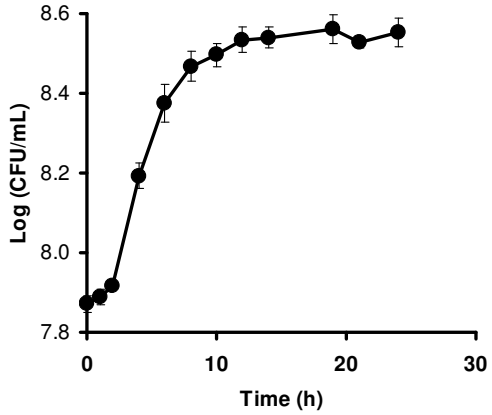

Fig 1. Growth curve of *Vibrio* sp. isolate MZ grown in seawater medium. The error bars represent mean ± standard deviation of triplicate data.

Table 3. Growth coefficients as modelled using the Huang model.

Parameter	Value	(95% confidence interval)	
A or Y_0 (Log CFU/mL)	0.324	0.278	0.370
μ_m (h^{-1})	0.322	0.252	0.392
lag (h)	2.683	2.030	3.337
Y_{max} (Log CFU/mL)	1.367	1.322	1.412

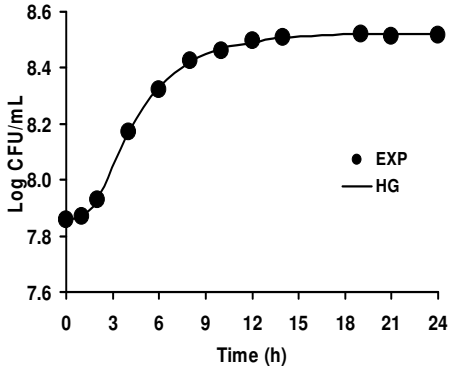

Fig. 2. Growth of *Vibrio* sp. isolate MZ as modelled using the Huang model.

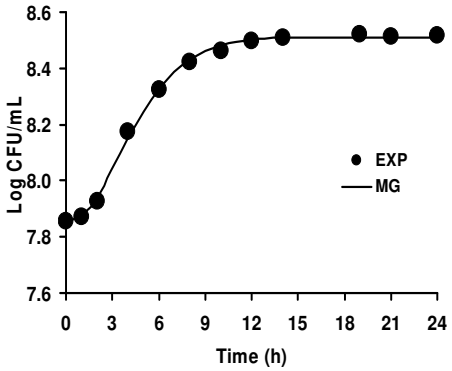

Fig. 3. Growth of *Vibrio* sp. isolate MZ as modelled using the modified Gompertz model.

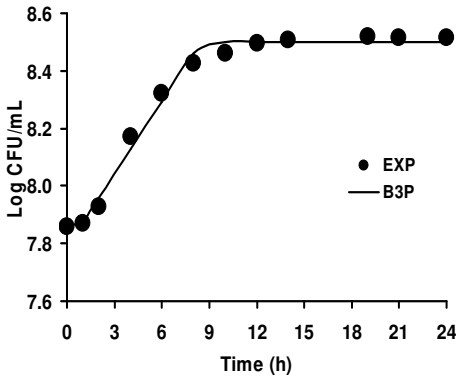

Fig. 4. Growth of *Vibrio* sp. isolate MZ as modelled using the Buchanan-3-phase model.

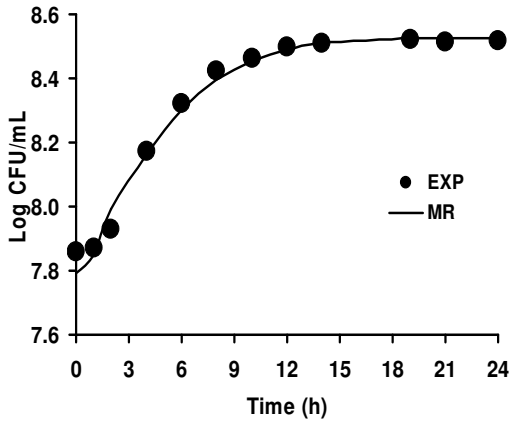

Fig. 5. Growth of *Vibrio* sp. isolate MZ as modelled using the modified Richard model.

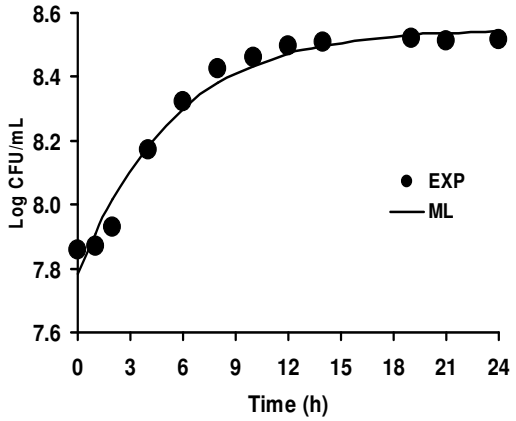

Fig. 6. Growth of *Vibrio* sp. isolate MZ as modelled using the modified Logistics model.

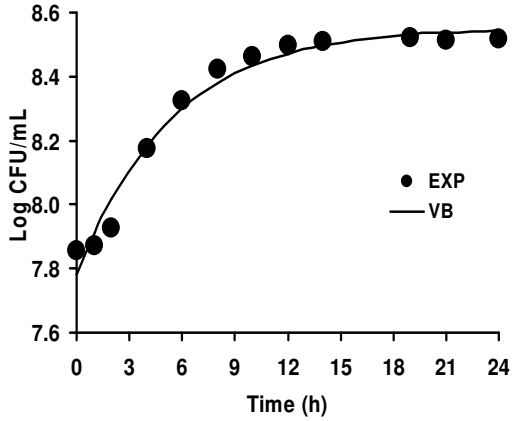

Fig. 7. Growth of *Vibrio* sp. isolate MZ as modelled using the von Bertalanffy model.

Table 2. Statistical tests for the various models utilized in modelling the growth curve of *Vibrio* sp. isolate MZ on seawater medium.

Model	*p*	RMSE	R^2	adR^2	AF	BF	AICc
Huang	4	0.01	1.00	1.00	1.00	1.00	-101.98
Baranyi-Roberts	4	0.05	0.98	0.97	1.00	1.00	-50.38
modified Gompertz	3	0.04	0.98	0.98	1.00	1.00	-62.50
Buchanan-3-phase	3	0.03	0.99	0.99	1.00	1.00	-68.41
modified Richards	4	0.04	0.99	0.98	1.00	1.00	-56.48
modified Schnute	3	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
modified Logistics	3	0.05	0.97	0.96	1.00	1.00	-56.62
von Bertalanffy	4	0.05	0.97	0.96	1.00	1.00	-56.04

Note: p is no of parameter

CONCLUSION

In conclusion, the Huang model was the best model in modelling the growth curve of a luminescent bacterium 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). This is the first time such a model has been found useful in modelling the growth curve of a luminescent bacterium. In addition, other secondary modelling works including the effect of environmental conditions (pH and temperature) on the growth parameters from this bacterium is ongoing.

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