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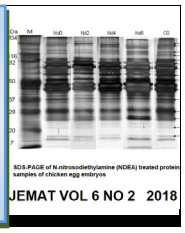

Mathematical Modelling of the Growth of *Caulobacter crescentus* on Caffeine

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ABSTRACT

Caffeine is a purine alkaloid naturally found in many species of plant and can be degraded by bacteria. Prolong caffeine consumption is well-known to have serious adverse effects. The used of linearization technique using natural logarithm transformation, though standard, is erroneous and can just give an estimated value for the sole parameter measured; the specific growth rate. In this paper, for the first time we present different kinetics models such as Von Bertalanffy, Baranyi-Roberts, modified Schnute, modified Richards, modified Gompertz, modified Logistics and most recent Huang were used to get values for the above constants or parameters from *Caulobacter crescentus* bacterium growth on caffeine. Huang model was found to be the best model with the highest adjusted R^2 value with the lowest RMSE value. The Accuracy and Bias Factors values were close to unity (1.0). The Huang parameters such as Y_{max} (bacterial growth upper asymptote), λ (lag time), μ_{max} (maximum specific bacterial growth rate) and A or Y_0 (bacterial growth lower asymptote) were found to be 1.367 (95% confidence interval of 1.322 - 1.412), 2.683 (95% confidence interval of 2.030 - 3.337), 0.322 (95% confidence interval of 0.252 - 0.392) and 0.324 (95% confidence interval of 0.278 - 0.370).

INTRODUCTION

Caffeine (1,3,7-trimethylxanthine) is a purine alkaloid naturally found in many species of plant. It is a major dietary ingredient in human found in common beverages and food products, such as guarana, kola nut, chocolates, tea, yerba mate, and coffee [1–3]. Caffeine can be degraded by many microorganisms such as bacteria and also through conventional method. Caffeine degradation using conventional techniques such as supercritical fluid and solvent extraction normally involve the use of solution containing aqueous coffee extract in addition to decaffeinating agents like supercritical CO_2, charcoal or carbon, ethyl acetate, and methylene chloride. These techniques are expensive, non-specific, and the chemicals are toxic to the environment thereby leading to the lost of flavor and aroma [4] . In recent years, the use of microorganisms such as bacteria is being studied as a potential method for decaffeination as it is specific, reduce time management, eco-friendly, economic, easier, disease free and cheaper [5,6] . Bacterial isolates capable of degrading caffeine has been studied in *Caulobacter crescentus,* Pseudomonas, Serratia, Leifsonia, Klebsiella among others. Unlike mammals, bacteria can use caffeine as a sole source of carbon and nitrogen for their growth [7,8] . Isolates with high caffeine growth capacity and competence to resist high caffeine concentrations are required for effective growth and degradation.

Usually, the growth curve of bacteria showed a sigmoidal pattern, starting with the lag section just after t = 0, followed by the logarithmic section and then the bacteria enters the stationary phase and finally moves to death phase or decline in bacterial growth. In order to describe the bacterial growth curve, various sigmoidal functions such as Von Bertalanffy, Baranyi-Roberts, modified Schnute, modified Richards, modified Gompertz, modified Logistics and stannard were compared [9]. They were compared statistically using a comprehensive model (Schnute model), which is a model that encompasses all other

models. The F test and the t test were used. In the F test, the lack of fit of the models is compared with the measuring error while in the t test, confidence intervals for parameters can be estimated and can be used to distinguish between the models. Furthermore, the models were compared with respect to their easy usage. In order to contain all biologically relevant parameters, all sigmoidal functions were modified. The models of Stannard, Schnute and Richards seemed to be essentially the same equation [10,11]. In the cases tested, the modified Gompertz equation was statistically adequate to explain the caffeine growth data. The growth curve valuable parameters are the maximum specific growth rate (μ_{max}), the lag period and the asymptotic values. The maximum specific growth rate (μ_{max}) value can be used in the development of secondary models to study the effects of substrate, temperature, pH and product on growth rate.

Most bacterial growth models lie between a mechanistic and empirical properties, though it is possible that these two categories exist in reality side by side [12] . In this finding, we present for the first time the use of primary models in modelling the *Caulobacter crescentus* growth curve.

Mathematical modelling of Mo-blue production have been explored previously [13,14] but all of these works utilize the linearization of the Mo-blue production over time profile to obtain the specific growth rate for further secondary modelling. As the benefits of nonlinear regression analysis of the Mo-blue production have been described above, thus, the objective of this work is to evaluate several available models such as Logistic [9,15], Gompertz [9,16], Richards [9,17], Schnute [9], Baranyi-Roberts [11], Von Bertalanffy [18,19], Buchanan three-phase [10] and more recently Huang model [12]. In this study, we show for the first time the applicability of the Huang model in modelling bacterial growth on caffeine.

MATERIALS AND METHODS

Data from Fig 1. from Gaul and Donegan [20] was processed using the software Webplotdigitizer 2.5 [21] which digitizes the scanned figure and has been utilized by many researchers and acknowledged for its reliability [22,23].

Statistical analysis

Statistical significant difference between the models was calculated through various methods including the adjusted coefficient of determination (R^2), accuracy factor (AF), bias factor (BF), Root-Mean-Square Error (RMSE) and corrected AICc (Akaike Information Criterion) as before [22].

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. μ_{max} of estimation was carried out by the steepest ascent rifle of the curve while the crossing of this line with the x-axis is an estimation of λ.

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

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	$y=A\left\{1+v\exp(1+v)\exp\left[\frac{\mu_m}{A}(1+v)\left(1+\frac{1}{v}\right)(\lambda-t)\right]\right\}^{\left(\frac{-1}{v}\right)}$
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	$y=A+\mu_m x+\frac{1}{\mu_m}\ln\left(e^{-\mu_m x}+e^{-h_0}-e^{-\mu_m x-h_0}\right)-\ln\left(1+\frac{e^{\mu_m x+\frac{1}{\mu_m}\ln\left(e^{-\mu_m x}+e^{-h_0}-e^{-\mu_m x-h_0}\right)}-1}{e^{(y\max-A)}}\right)$
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	$y=A+y\max-\ln\left(e^A+\left(e^{Y\max}-e^A\right)e^{-\mu_m B(x)}\right)$ $B(x)=x+\frac{1}{\alpha}\ln\frac{1+e^{-\alpha(x-\lambda)}}{1+e^{\alpha\lambda}}$
Buchanan Three-phase linear model	3	Y = A, 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_{max}= 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 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**.

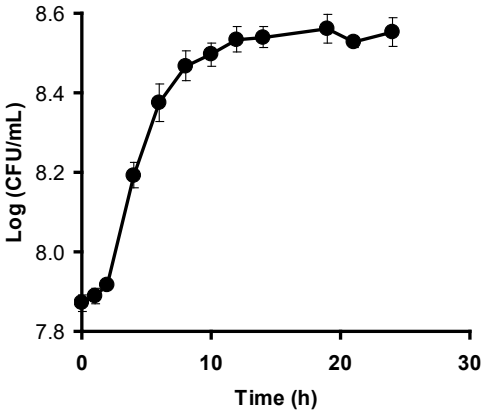

Fig 1. Growth curve of *Caulobacter crescentus*. The error bars represent mean ± standard deviation of triplicate data.

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

Parameter	Value	(95% Confidence interval)
A or Y_0 (ln OD_{600} nm)	0.324	0.278–0.370
μ_{max} (h^{-1})	0.322	0.252–0.392
lag (h)	2.683	2.030–3.337
Y_{max} (ln OD_{600} nm)	1.367	1.322–1.412

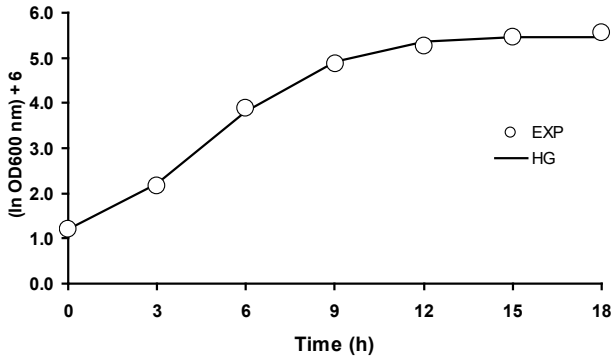

Fig. 2. Growth of *Caulobacter crescentus* as modelled using the Huang model.

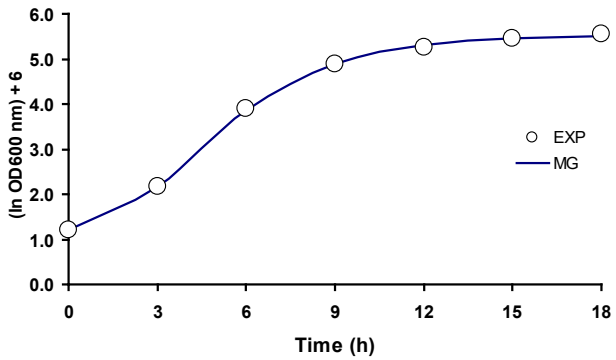

Fig. 3. Growth of *Caulobacter crescentus* as modelled using the modified Gompertz model.

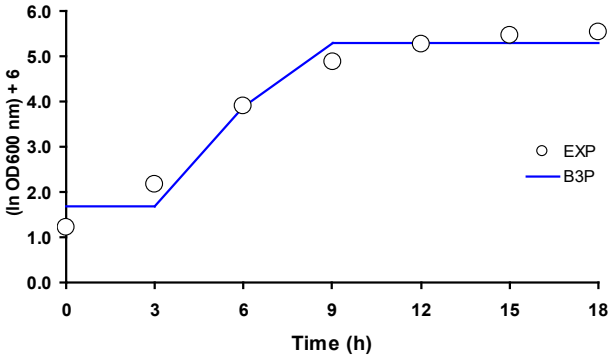

Fig. 4. Growth of *Caulobacter crescentus* as modelled using the Buchanan-3-phase model.

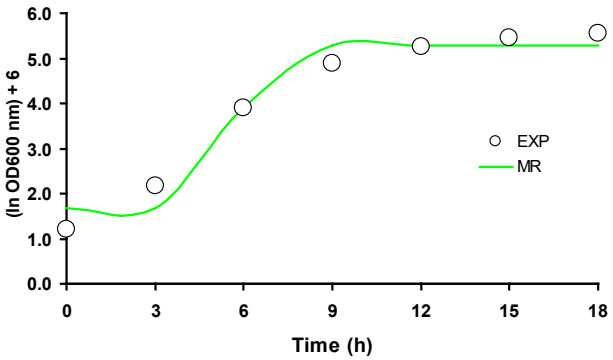

Fig. 5. Growth of *Caulobacter crescentus* as modelled using the modified Richard model.

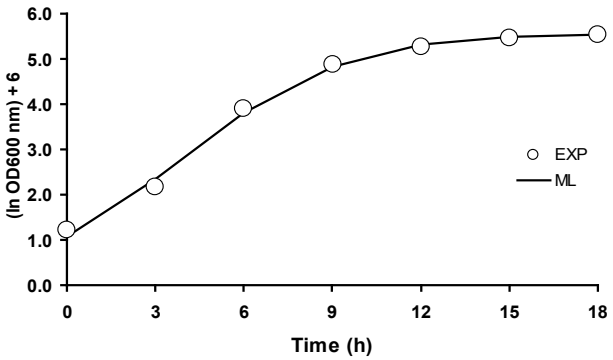

Fig. 6. Growth of *Caulobacter crescentus* as modelled using the modified Logistics model.

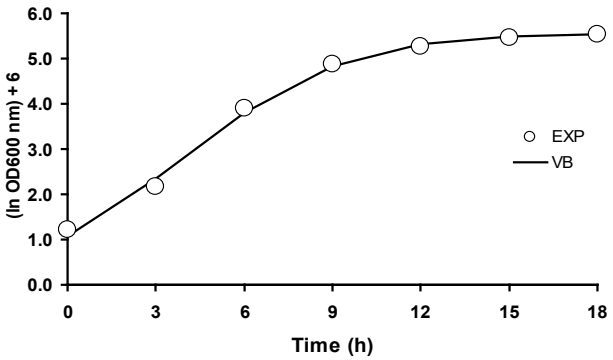

Fig. 7. Growth of *Caulobacter crescentus* as modelled using the von Bertalanffy model.

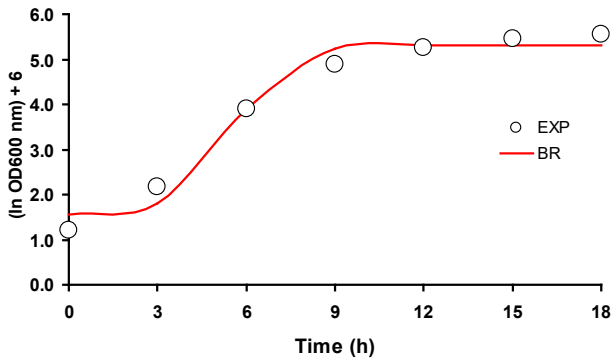

Fig. 8. Growth of *Caulobacter crescentus* as modelled using the Baranyi-Roberts model.

Table 2. Statistical tests for the various models utilized in modelling the growth curve of *Caulobacter crescentus*.

Model	*p*	RMSE	adR^2	AF	BF	AICc
Huang	4	0.09	1.00	1.01	1.00	38.62
Baranyi-Roberts	4	0.39	0.92	1.09	1.01	58.94
modified Gompertz	3	0.33	0.95	1.11	1.00	14.73
Buchanan-3-phase	3	0.42	0.92	1.11	1.01	18.08
modified Richards	4	0.11	0.99	1.02	1.00	40.69
modified Schnute	3	n.a.	n.a.	n.a.	n.a.	n.a.
modified Logistics	3	0.12	0.99	1.03	0.99	0.21
von Bertalanffy	4	0.25	0.97	1.07	0.99	10.64

Note: p is no of parameter

The Huang model gave the best fitting based on statistical test with the lowest values for RMSE the highest value for adjusted R^2 and the closest values to unity for both Accuracy and Bias factors. However, the corrected Akaike Information Criteria was best for the Baranyi-Roberts model. The poorest performance was modified Schnute which failed to converge.

The Huang model was only recently introduced [24], but has found applications in modelling bacterial growth in various substrates such as the growth of *Pseudomonas* spp. in pallet-package pork at 10 ºC [25], the growth of *Phyllosticta citricarpa* McAlp Van der Aa; the citrus black spot disease [26] and modelling the growth of *Klebsiella pneumoniae* on 2-methylquinoline [27].

Parameters obtained from the fitting exercise were maximum growth rate (μ_{max}), lag time (□), maximal growth (Y_{max}) and minimal growth (Y_o). These biologically meaningful coefficients will especially maximum growth rate is useful for secondary modelling exercise using more complex "secondary models" such as Haldane, Aiba, Yano, and others which will give us information such as the effect of substrate on growth rate [2]. The modelling shows that caffeine is toxic to bacterial growth (**Table 3**) resulting in a decrease in the maximum growth attained as the concentration of caffeine was increase **(Fig. 9).** The lag period was not severely affected suggesting that probably the cells was able to overcome the toxicity of caffeine at the beginning of growth. However, the growth rate was found to decrease (**Table 4**), which indicates the cellular growth process is affected by caffeine.

Caffeine is an important constituent in coffee and tea, which reveals the stimulatory effect to these beverages. Apart from the stimulatory effects, prolong caffeine consumption is linked with many health effects such as osteoporosis, cardiac arrhythmias, apathy, fatigue, adrenal stimulation, gastrointestinal complications, change in blood sugar among others [28–30]. Excessive caffeine intake also leads to increase in fetus malfunction and infertility during pregnancy, complication in aging, heart disease and major cause of cancer [31,32]. Apart from the deleterious caffeine health effects, caffeine degradation is paramount from an environmental point of view. Tea and coffee industries produced solid wastes such as tea waste, husk, and coffee pulp, for which caffeine is one of the major toxic compounds. Although, these wastes are enriched with proteins and carbohydrates, they cannot be used as animal feed due to the occurrence of caffeine and other toxic compounds [8,33,34]. The caffeine in water wastes of tea and coffee industries cannot be allowed to be channeled into seas and waterways as it would affect the marine environment [35,36]. Therefore, caffeine degradation is of paramount in view of health as well as general environmental concerns.

Table 4. The growth parameters for *Caulobacter crescentus* grown on caffeine as modelled using the Huang model.

Parameters	0 mM			0.331 mM		0.66 mM		1.3 mM		2.65 mM		5.3 mM	
Y_0	1.22	±	0.09	0.99	±0.05	0.70	± 0.07	0.69	± 0.15	0.69	± 0.04	0.28	±0.10
Lag	1.28	±	0.28	0.60	±0.21	0.47	± 0.29	0.34	± 0.12	0.63	± 2.42	0.24	±0.66
Y_{max}	5.47	±	0.06	5.46	±0.04	5.42	± 0.05	5.31	± 0.10	5.26	± 0.03	4.81	±0.13
$\mu_{max\ max}$	0.59	±	0.04	0.55	±0.02	0.59	± 0.03	0.57	± 0.06	0.45	± 0.01	0.36	±0.02

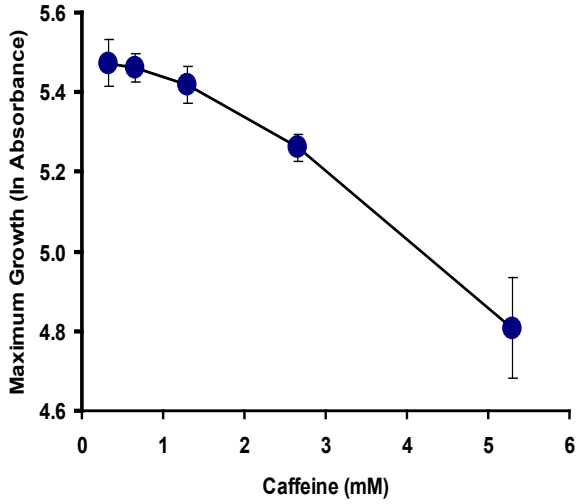

Fig. 9. The effect of caffeine concentration on the maximum growth attained by *Caulobacter crescentus* as modelled using the Huang model.

In basic research, these mechanistic models are used and are meant to reach a better understanding of the biological, chemical and physical processes that lead to the growth profile seen. All other things being equal, mechanistic models are more powerful since they tell you about the fundamental procedures driving patterns. They are more probable to work properly when concluding beyond the observed conditions [37].

CONCLUSION

In conclusion, The Huang model was the best model in modelling the *Caulobacter crescentus* growth curve on caffeine based on statistical tests such as corrected AICc (Akaike Information Criterion), bias factor (BF), adjusted coefficient of determination (R^2) and root-mean-square error (RMSE). Parameters obtained from the fitting exercise were maximum growth rate (μ_{max}), lag time (λ), maximal growth (Y_{max}) and

minimal growth (Y_o). The use of bacterial growth models to obtained exact growth rate is advantageous for further development of secondary model and this work has revealed the capability of such models. Current findings comprise secondary modelling of the growth rate from this bacterium specifically on the caffeine inhibitory effect on the maximum growth rate values obtained from this works.

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