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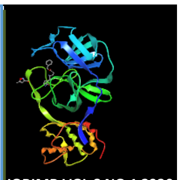

JOBIMB VOL 8 NO 1 2020
SARS-Cov-2 protease

Modelling the Growth of *Enterobacter* sp. on Polyethylene

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HISTORY

Received: 25th March 2020
Received in revised form: 14th of April 2020
Accepted: 18th of May 2020

KEYWORDS

Polyethylene
Entrobacter sp
Mathematical modelling
growth
Huang

ABSTRACT

Although standard, the use of linearization techniques using natural logarithm transformation is erroneous and can only give an estimated value for the measured parameter; the specific growth rate. For the first time, in this paper, we present different kinetic models such as Von Bertalanffy, Baranyi-Roberts, modified Schnute, modified Richards, modified Gompertz, modified Logistics and most recent Huang were employed to obtain values for the above constants or parameters from *Enterobacter* sp. growth on polyethylene. Huang model was found to be the best model with the highest adjusted R^2 value and 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

The growth curve of bacteria typically showed a sigmoidal pattern, beginning with the lag section just after t = 0, followed by the logarithmic section and then the bacteria reach the stationary phase and finally enters the death phase or decline in bacterial growth. To describe the bacterial growth curve, various sigmoidal functions such as Von Bertalanffy, Baranyi-Roberts, Schnute modified, Richards modified, Gompertz modified, Logistics modified, and Stannard were compared [1]. They were statistically compared using a comprehensive model (Schnute model), a model which encompasses 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 can be estimated for parameters and be used to distinguish between the models. In addition, the models were compared with respect to their ease of use. All sigmoidal functions were modified to include all of the biologically relevant parameters. The Stannard, Schnute and Richards models seemed to be having the same equations [2,3]. Of all the cases tested, the modified Gompertz equation was statistically sufficient to explain the polyethylene growth data. The valuable parameters of the growth curve are the maximum specific growth rate (μ_{max}), the lag period and asymptotic values. In the development of secondary models, the maximum growth rate (μ_{max}) value can be used in order to study the effects of substrate, temperature, pH and product on the growth rate. Most models of bacterial growth lie between mechanistic and empirical properties, although these two groups can exist side by side in reality (4). In this finding, the use of primary models in the modelling of *Entrobacter* sp. growth curve on polyethylene is presented for the first time. As the benefits of nonlinear regression analysis have been described above, therefore, this studies is aimed at evaluating several available models such as Logistic [1,5], Gompertz [1,6], Richards [1,7], Schnute [1], Baranyi-Roberts [3], Von Bertalanffy [8,9], Buchanan three-phase [2] and more recently Huang model [4]. For the first time, the applicability of the various model in modelling bacterial growth on polyethylene is demonstrated.

MATERIALS AND METHODS

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;
Y_{max}= Bacterial growth upper asymptote;
μ_{max}= maximum specific bacterial growth rate;
v= affects near which asymptote maximum growth occurs.
λ=lag time
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}) or (d^{-1}) can be calculated as h_0=μ_{max}

Data acquisition

The graphical data of a published work by Ren et al [10] from Fig 2a. (changes in the percentage of carbon and oxygen atoms on the PE film surface after a 31-day incubation) was processed using the software Webplotdigitizer 2.5 [11] which digitizes the scanned figure and has been use and acknowledged by many researchers due to its precision and reliability [12,13].

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 [12].

Fitting of the data

Fitting of the bacterial growth curve using various growth models (**Table 1**) was performed by the use of CurveExpert Professional software (Version 1.6) through nonlinear regression utilizing the Marquardt algorithm. The maximum growth rate (μ_{max})) estimation was obtained from the steepest ascent rifle of the curve while the lag time (λ) estimation was done using the line crossing the X-axis. The highest growth was selected for the modelling exercise.

RESULTS AND DISCUSSION

All of the curves tested show visually acceptable fitting (**Figs 2** to **8**). Growth data should be converted to log unit before modelling is carried out. The best performance was the Buchanan-3-phase 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 the modified Richard model (**Table 2**). The coefficients for the Buchanan-3-phase model are shown in **Table 3**.

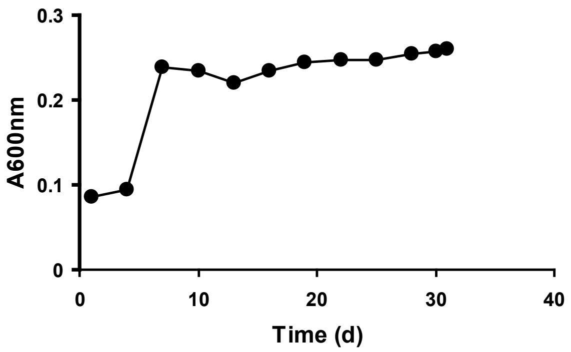

Fig 1. Replotted data on the growth of *Enterobacter* sp. on polyethylene.

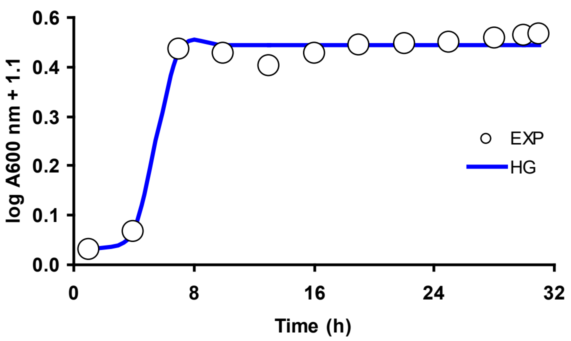

Fig. 2. Growth of *Enterobacter* sp. on polyethylene as modelled using the Huang model.

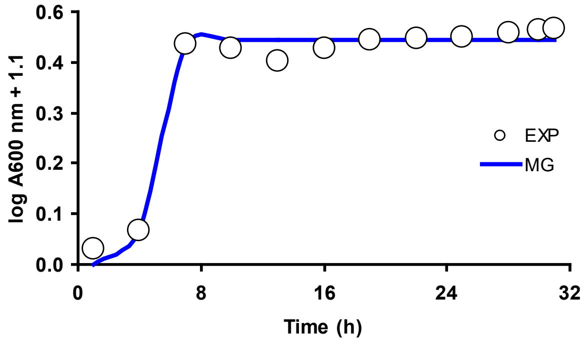

Fig. 3. Growth of *Enterobacter* sp. on polyethylene as modelled using the modified Gompertz model.

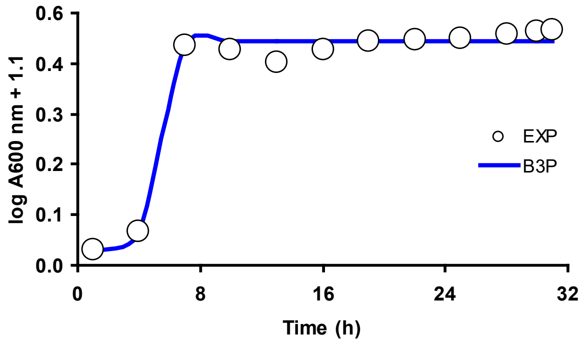

Fig. 4. Growth of *Enterobacter* sp. on polyethylene as modelled using the Buchanan-3-phase model.

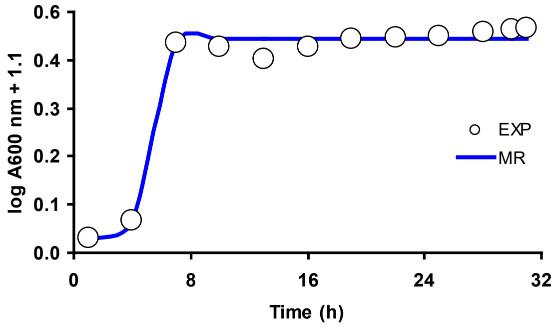

Fig. 5. Growth of *Enterobacter* sp. on polyethylene as modelled using the modified Richard model.

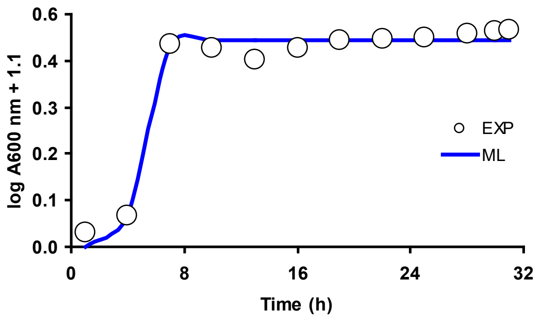

Fig. 6. Growth of *Enterobacter* sp. on polyethylene as modelled using the modified Logistics model.

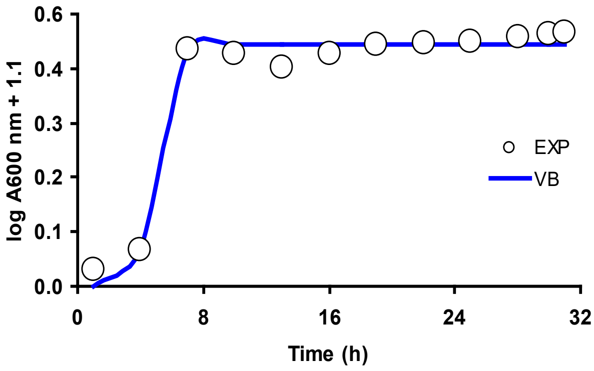

Fig. 7. Growth of *Enterobacter sp*. on polyethylene as modelled using the von Bertalanffy model.

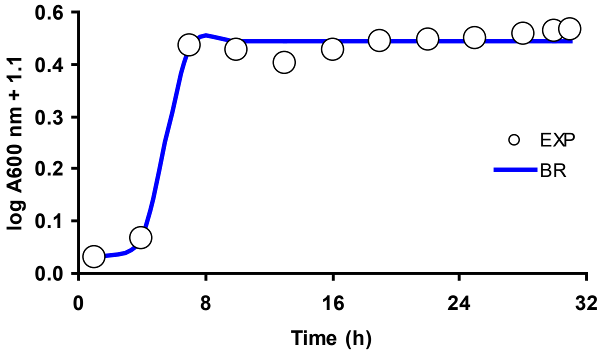

Fig. 8. Growth of *Enterobacter sp*. on polyethylene as modelled using the Baranyi-Roberts model.

Table 2. Statistical tests for the various models utilized in modelling the growth curve of *Enterobacter* sp. on polyethylene.

Model	*p*	RMSE	R^2	AdR^2	AF	BF	AICc
Huang	4	0.023	0.987	0.979	1.028	1.001	-67.71
Baranyi-Roberts	4	0.023	0.987	0.979	1.028	1.001	-67.69
modified Gompertz	3	0.024	0.984	0.979	1.032	1.001	-73.00
Buchanan-3-phase	3	0.022	0.987	0.982	1.032	0.990	-75.69
modified Richards	4	0.026	0.984	0.975	1.028	1.001	-64.71
modified Schnute	3	0.043	0.952	0.924	1.087	1.037	-52.33
modified Logistics	3	0.024	0.984	0.979	1.028	1.001	-73.05
von Bertalanffy	4	0.046	0.935	0.911	1.105	1.053	-57.51

Note: p is no of parameter

Table 3. Growth coefficients as modelled using the Buchanan-3-phase model.

Parameters	Value	95% Confidence interval
μ_{max}	0.135	0.110 to 0.159
Lag (h)	3.704	3.124 to 4.284
y_{max} (A600nm)	0.244	0.234 to 0.254

The choice of the Buchanan as the best model is apt since the model is the simplest amongst the eight and it is a three-parameter model giving it a higher degree of freedom compared to four- or five-parameter models. This is important when a growth curve having a smaller number of points is used. In addition all three parameters have biological meaning due to the highly mechanistic property of the model. The Buchanan three-phase model has been successfully used to model growth of bacteria [3,14,16], algae [17] and worms [18].

Nonlinear regression of the Baranyi-Roberts model could be problematic in some cases as it is rather sensitive to the number and distribution of data points [2,3]. Buchanan et al. [2] developed a simpler three-phase linear model to overcome this problem.
The assumptions of the Buchanan model were as follows;

(i) that the specific growth rate is equal to zero during the lag phase,
(ii) the logarithm of the bacterial cells increases linearly with respect to time during the exponential phase and
(iii) the specific growth rate is zero during the stationary phase.

These assumptions can be expressed as follows;

Lag Phase:
for $t \leq t_{lag}$,
$N_t = N_o$

Exponential growth phase:
For $t_{lag} < t < t_{max}$,
$N_t = N_o + \mu(t - t_{lag})$

Stationary phase:
For $t \geq t_{max}$,
$N_t = N_{max}$

Where N_o = Log of initial population density (optical density) or bacterial cell number (CFU/ml); N_t = Log population density (optical density) or bacterial cell number (CFU/ml) at time t; N_{max} = Log of the maximal population density (optical density) or bacterial cell number (CFU/ml); t = elapsed time (h); t_{max} = time (h) when the maximum population density (optical density) or bacterial cell number (CFU/ml) is reached; t_{lag} = time (h) at the end of the lag phase and μ = specific growth rate (log (CFU/ml)/h).

The Buchanan model greatest advantage is its straightforwardness. Additionally, it supplies an approximation to the mathematical means microbiologists have usually used to calculate growth kinetic graphically [2]. Its disadvantage include the fact that it could only fit growth curves having an abrupt transition from the lag phase to exponential phase [19].

It has been suggested that when a three-parameter model is sufficient to describe the data, experts recommend over a four-parameter model given that the three-parameter model is much simpler and as a consequence much easier to use and solution is more stable considering that the parameters are much less correlated. On top of that, every time a three-parameter model is employed, the estimates have more degrees of freedom, and this can be crucial every time a growth curve or generation curve with a small number of measured points is employed. In addition, it is essential that all three parameters may be given a biological interpretation.

Parameters obtained from the fitting exercise were maximum growth rate (μ_{max}), lag time (λ) and maximal bacterial growth (Y_{max}). These three biologically meaningful coefficients can be later be used for secondary modelling of fish growth using model such as the two-parameter Monod model or other more complex models "secondary models" such as Haldane, Aiba, Yano and others. Such mechanical models are used in fundamental research to better understand the physical, chemical and biological processes leading to the observed growth profile.
All others, mechanical models are more important because they inform you about the driving trends of the observed phenomena. They function best when extrapolating beyond the conditions they are seen [20].

CONCLUSION

In conclusion, the Buchanan-3- phase model was found to be the best model in modelling the growth of *Entrobacter* sp. on polyethylene based on the statistical tests such as corrected AICc (Akaike Information Criterion), bias factor (BF), adjusted coefficient of determination (R^2) and root-mean-square error (RMSE) carried out. 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 obtain an exact growth rate is advantageous for further development of secondary model and this work has demonstrated the capability of such models.

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