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Modelling the Growth Kinetics of *E. coli* Measured Using Real-Time Impedimetric Biosensor

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The development of in situ sensor for measuring bacterial concentrations in fermenter would allow real-time monitoring of the concentration of bacteria. Kim et al [1] has developed such a method using impedance spectroscopy, and was able to measure in real-time the concentration of *E. coli* at 0.01 MHz frequency using impedance changes. In this work we used several mathematical models of bacterial growth kinetics such as logistic, Gompertz, Richards, Schnute, Baranyi-Roberts, Von Bertalanffy, Buchanan three-phase and the Huang models to model the resulting bacterial growth curve from Kim et al. The Buchanan three-phase 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). Parameters obtained from the growth fitting exercise were maximum specific growth rate (μ_{max}), lag time (λ) and maximal number of cells achieved per droplet (Y_{max}) with values of 0.67±0.086 (h^{-1}), 2.45±0.24 (h) and 20.26±0.038 (ln cell no/ml), respectively. The parameters obtained from fitting the bacterial growth curve using this model can be used for further modeling and optimization exercises for identifying key optimal parameters for improving the sensitivity of the biosensor.

Growth curves are found in a wide range of disciplines, such as fishery research, crop science, and biology. Most living matter grows with successive lag, growth, and asymptotic phases; examples of quantities that follow such growth curves are the length or mass of a human, a potato, or a fish and the extent of a population of fish or microorganisms. One of the most important results from curve fitting in growth curve model is the ability to use a growth model that have a strong underlying mechanistic function based on sound theoretical knowledge of the system (2,3). One of the best of such model is the Michaelis-Menten kinetics that models the effect substrate on the initial enzyme activity of the enzyme, substrate composition, temperature, light, pH, and genetic.

Monitoring bacterial growth has been traditionally carried out using plate count agar or through counting on a haemocytometer. These methods are time consuming, require trained personnel and cannot be carried out in real-time. Due to this, several biosensor-based methods have been develop to overcome these hurdles including impedemetric biosensor. Impedance spectroscopy utilizes electrical properties of materials and their interfaces with electronically conducting electrodes. It is a relatively novel and powerful method (1,4,5). The use of this method by Kim et al. (1) for monitoring bacterial growth has been explored and showed promising results. The resultant bacterial growth showed a unique sigmoidal characteristics of bacterial growth including a lag time (λ) followed by an acceleration to a maximal value (μ_{max}) or exponential phase culminating in a final phase in which the rate decreases and finally reaches zero, so that an asymptote (A) is reached (6).

The sigmoidal curve can be fitted by different mathematical functions, such as the Logistic (6,7), Gompertz (6,8), Richards (6,9), Schnute (6,10), Baranyi-Roberts (2) and Von Bertalanffy (11), Buchanan three-phase (12) and more recently Huang models (13).

Table 1. Growth models used in this study

Model	No. of parameter	Equations
Modified Logistic	3	$y = \frac{A}{\left\{1+\exp\left[\frac{4\mu_{max}}{A}(\lambda - t)+2\right]\right\}}$
Modified Gompertz	3	$y = A\exp\left\{-\exp\left[\frac{\mu_{max}e}{A}(\lambda - t)+1\right]\right\}$
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	$y = A+\mu_{max}x+\frac{1}{\mu_{max}}\ln\left(e^{-\mu_{max}x}+e^{-h_0}-e^{-\mu_{max}x-h_o}\right)$ $-\ln\left(1+\frac{e^{\mu_{max}x+\frac{1}{\mu_{max}}\ln\left(e^{-\mu_{max}x}+e^{-h_0}-e^{-\mu_{max}x-h_0}\right)}-1}{e^{(y_{max}-A)}}\right)$
Von Bertalanffy	3	$y=K\left[1-\left[1-\left(\frac{A}{K}\right)^3\right]\exp^{-\left(\mu_{max}x/3K^{\frac{1}{3}}\right)}\right]^3$
Huang	4	$y = A+y_{max}-\ln\left(e^{A}+\left(e^{Y_{max}}-e^{A}\right)e^{-\mu_{max}B(x)}\right)$ $B(x)=x+\frac{1}{\alpha}\ln\frac{1+e^{-\alpha(x-\lambda)}}{1+e^{\alpha\lambda}}$
Buchanan Three-phase linear model	3	$y = A$, if $x < lag$ $y = A+k(x-\lambda)$, if $\lambda \le x \ge x_{max}$ $y = y_{max}$, if $x \ge x_{max}$

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;
t = sampling time
a,b,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 $h_0 = \mu_{max}$

Materials and Methods

Acquisition of Data

In order to process the data, the *E. coli* growth curve from Figure 8 from Kim et al (1) was scanned and electronically processed using WebPlotDigitizer 2.5 (Rohatgi, 2014) which helps to digitize scanned plots into table of data with good enough precision (De Stefano et al., 2014; Kivlin et al., 2013). Data were then replotted (Fig. 1).

Fitting of the data

Growth data will be fitted nonlinearly using nonlinear regression software (CurveExpert Professional software, Version 1.6). The method uses the Marquardt algorithm which minimizes the sums of square of residuals between the predicted and experimental values. The program can be used in the manual mode through manual input of values or automatic mode where it calculates starting values by searching for the steepest ascent of the curve. This is normally done using four datum points to estimate the μ_{max}. The intersection of this line with the x axis is the estimation value of the lag time or λ while the final datum point is the estimation of 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 (R2), bias factor (BF), accuracy factor (AF) and corrected AICc (Akaike Information Criterion).

The RMSE was calculated according to Eq. (1), where Pdi are the values predicted by the model and Obi 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).

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

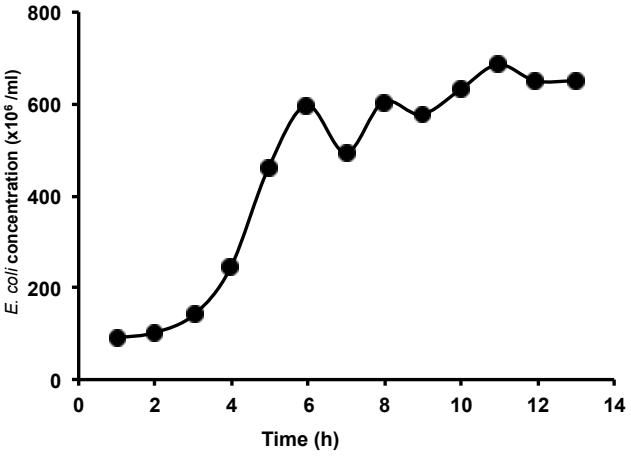

Figure 1. Growth curve of *E. coli* as measured using impedance spectroscopy (Replotted from Kim et al).

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

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

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 model to another is normal, the adoption of the method does not readily provides comparable analysis. Hence an adjusted R^2 (Eq. 2 and 3) is used to calculate the quality of nonlinear models according to the formula where RMS is Residual Mean Square andis the total variance of the y-variable (14).

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 is 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 (16). The AICc is calculated for each data set for each model according to the following equation (Eq. 4);

$$AICc = 2p + n\ln\left(\frac{RSS}{n}\right) + 2(p+1) + \frac{2(p+1)(p+2)}{n-p-2} \quad (4)$$

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 (16,17).

Accuracy Factor (AF) (Eq. 5) and Bias Factor (BF) (Eq. 6) to test for the goodness-of-fit of the models as suggested by Ross (18) 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 > 1indicates a fail-safe model. The Accuracy Factor is always ≥ 1, and higher AF values indicate less precise prediction.

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

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

Results and Discussions

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 the eight only Modified Gompertz, Huang, Baranyi-Roberts and Buchanan three-phase models (Figs. 2-5) could model the *E. coli* growth curve.

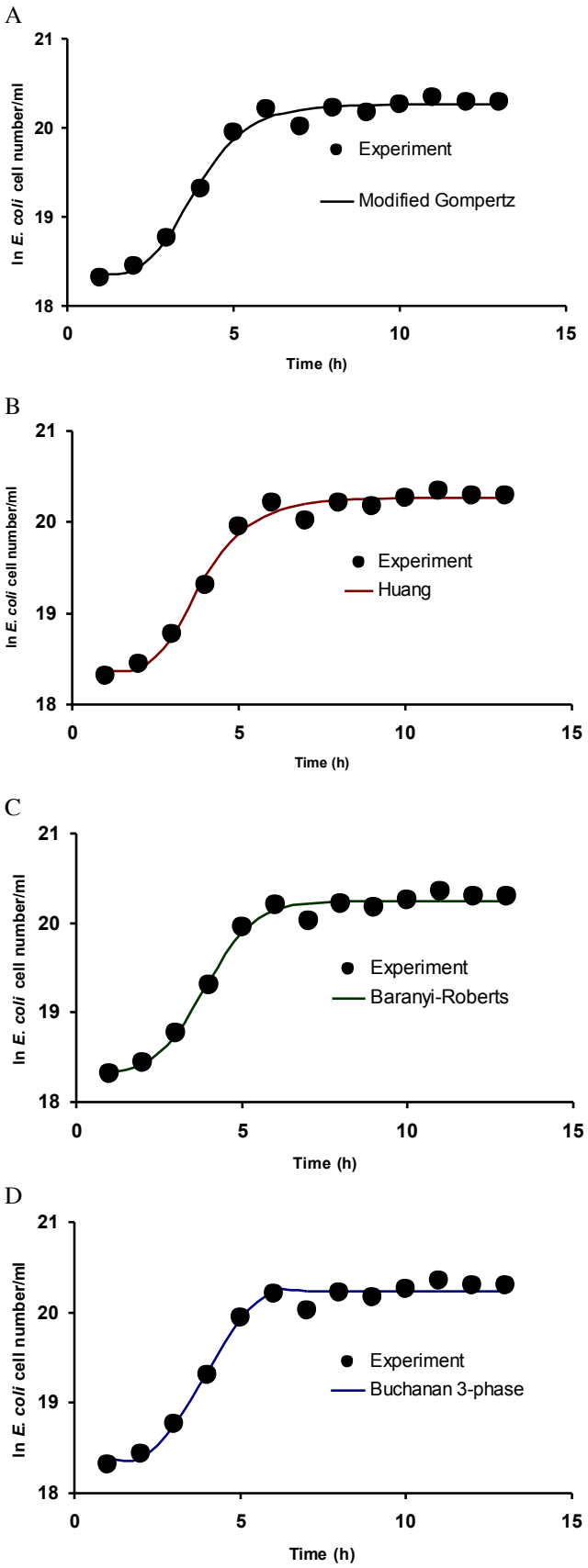

Figure 2. Growth curve of *E. coli* fitted with Gompertz (A), Huang (B), Baranyi-Roberts (C), and Buchanan three-phase growth model (D). The number of cells/ml was transformed into natural logarithm.

Table 2. Statistical analysis of the various fitting models.

Model	*p*	SSE	MSE	RMSE	AICc	Ra2	BF	AF
Modified Gompertz	3	0.0784	0.0060	0.0777	0.986	-67.46	1.0000	1.0032
Huang	4	0.0840	0.0070	0.0837	0.983	-59.98	1.0000	1.0034
Baranyi-Roberts	4	0.0715	0.0060	0.0772	0.986	-62.58	0.9999	1.0028
Buchanan	3	0.0812	0.0062	0.0790	0.985	-66.90	1.0001	1.0030

p	no of paramaters
SSE	Sums of Square Error
MSE	Mean Square Error
RMSE	Root Mean Square Error
AICc	Corrected Akaike Information Criterion
Ra^2	Adjusted coefficient of determination
BF	Bias factor
AF	Accuracy factor

The results indicate that all of the models used could fit the growth curves. The modified Gompertz model was chosen as the best model based on the lowest AICc and highest adjusted R^2 values (Table 2). Parameters obtained from the growth fitting exercise were maximum specific growth rate (μ_{max}), lag time (λ) and maximal number of cells achieved per droplet (Y_{max}) with values of 0.67±0.086 (h^{-1}), 2.45±0.24 (h) and 20.26±0.038 (Ln cell no/ml), respectively.A close contender is the four-parameter Baranyi-Roberts model. The Gompertz model is a three-parameter one, whereas the Huang and Baranyi-Roberts model are four-parameter models. Three-parameter model is more stable and is simpler and easier to use and the parameters are less correlated (6,17). In addition, a three-parameter model has more degrees of freedom for the parameter estimates. Furthermore, all three parameters can be given a biological meaning. In contrast, the fourth parameter in the four-parameter model is a shape parameter and is usually difficult to assign any biological and physical meanings (6,17,19). This means that the Gompertz model should be more appropriate than the other models in describing the growth kinetics of this bacterium.

Conclusion

In conclusion, several of the sigmoidal functions evaluated can be used to model *E. coli* growth rate from an impidemetric biosensor set-up with the best model is the modified Gompertz with an acceptable degree of goodness- of-fit. The parameters obtained from the growth curve using this model can be used for further modeling and optimization exercises for identifying key controlling parameters of the biosensing device.

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