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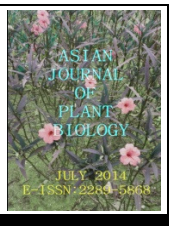


# Modeling the Growth Kinetics of *Chlorella vulgaris* Cultivated in Microfluidic Devices

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The third generation biofuels such as algal biodiesel is the future potential source of renewable energy. A recent develpoment of a drop-based microfluidics device platform to investigate cellular growth kinetics of single and few cells of *Chlorella vulgaris* shows promising results for miniaturization of biodiesel screening. The results showed the typical asymmetric sigmoidal growth pattern. Since there exists a variety of models for describing the growth profile of microorganism such as logistic, Gompertz, Richards, Schnute, Baranyi-Roberts, Von Bertalanffy, Buchanan three-phase and more recently Huang models, the growth curves exhibit under such conditions would be an excellent study for finding the best model. 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), corrected AICc (Akaike Information Criterion) and F-test. Parameters obtained from the growth fitting exercise were maximum specific growth rate ($\mu_{max}$), lag time ($\lambda$) and maximal number of cells achieved per droplet ($Y_{max}$) with the values of 1.301 ($day^{-1}$), 1.861 (day) and 77 (no of cells/droplet), respectively. The parameters obtained from fitting the algae growth curve using this model can be used for further modeling and optimization exercises for identifying key controlling parameters of the microfluidic devices.

## INTRODUCTION

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. 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.

Algae growth often shows a phase in which the specific growth rate starts at a value of zero and then accelerates to a maximal value ($\mu_{max}$) in a certain period of time, resulting in a lag time ($\lambda$). 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.

The sigmoidal curve can be fitted by different mathematical functions, such as the Logistic [1,2], Gompertz [2,3], Richards [2,4], Schnute [2,5], Baranyi-Roberts [6] and Von Bertalanffy [7,8], Buchanan three-phase [9] and more recently Huang models [10]. Apart from demonstrating predictive ability and internal consistency, which is a must, the usefulness of a model should also be judged by its mathematical simplicity, flexibility, the number of its adjustable parameters and, where appropriate, whether they have intuitive meaning. The objective of this work is to evaluate similarities and differences between the models using published available data that lacks modeling and to deal with the question of which model(s) can be used, on the basis of statistical reasoning. This should give new data and results that could spurn

further information and improvement in the works already done by researchers.

Table 1. Growth models used in this study.

| Model | n | Equation |
|---|---|---|
| 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 | $y = K\left[1-\left[1-\left(\frac{A}{K}\right)^3\right]\exp^{-\left(rx/3K^{\frac{1}{3}}\right)}\right]^3$ |
| Huang | 4 | $y = A+y_{\max}-\ln\left(e^A+\left(e^{y_{\max}}-e^A\right)e^{-\mu_{\max}B(x)}\right)$ <br> $B(x) = x+\frac{1}{\alpha}\ln\frac{1+e^{-\alpha(x-\lambda)}}{1+e^{\alpha\lambda}}$ |
| Buchanan Three-Phase Linear Model | 3 | Y = A, IF X < LAG <br> Y=A + *K*(X–λ), IF λ ≤ X ≥ $X_{MAX}$ <br> Y = $Y_{MAX}$, IF X ≥ $X_{MAX}$ |

Note:
a= bacterial lower asymptote;
$\mu_{max}$= 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
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 $h_0=\mu_{MAX}$

## MATERIAL AND METHODS

### Acquisition of Data

In order to process the data, the graph from Figure 6A showing *Chlorella vulgaris* growth profile starting from one cell per droplet [11] were scanned and electronically processed using WebPlotDigitizer 2.5 [12] which helps to digitize scanned plots into table of data with good enough precision [13,14]. 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) that uses the Marquardt algorithm. This algorithm minimizes the sums of square of residuals between the predicted and experimental values. The program can be used in the manual mode or automatic mode where it calculates starting values by searching for the steepest ascent of the curve normally using four datum points to estimate the μmax. 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 ($R^2$), bias factor (BF), accuracy factor (AF), corrected AICc (Akaike Information Criterion) and F-test [15].

The RMSE was calculated according to Eq. (2), 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.

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

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 model 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.

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

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

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 [16].

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 [15].

The F-test is a statistic test used to find the most significant model between available predicted curve-fitting models. The analysis procedure includes selecting the model with the smallest RSS among all the models with the same or different number of fitting parameters followed by comparing the relative value of the F-ratio. In the event the F-ratio of the two models surpasses the upper quartile, the better complicated model is accepted as statistically significant [15]. Equation 5 is for models with same number of parameters while Equation 6 is for models with different number of parameters.

Accuracy Factor (AF) and Bias Factor (BF) to test for the goodness-of-fit of the models as suggested by Ross [17] were also used. The Bias Factor equal to1 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.

$$F = \frac{SS_1}{SS_2} \quad (5)$$

$$F = \frac{(SS_1 - SS_2)/(df_2 - df_1)}{SS_2 / df_2} \quad (6)$$

$$\text{Bias factor} = 10^{\left(\sum_{i=1}^{n} \log \frac{(Pd_i / Ob_i)}{n}\right)} \quad (7)$$

$$\text{Accuracy factor} = 10^{\left(\sum_{i=1}^{n} \log \frac{|(Pd_i / Ob_i)|}{n}\right)} \quad (8)$$

## 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). Buchanan three-phase, Baranyi-Roberts and Huang gave the best fitting based on statistical test with similar values for all statistical tests with the exception of the AICc test where the Buchanan model gave the best results of the three and hence was chosen as the best model. The poorest performance was Von Bertalanffy with the lowest score for all statistical tests (Table 2). The Buchanan three-phase model incidentally gave the most significant results as the more accurate model based on the F-test results in comparison with each of the models tested.

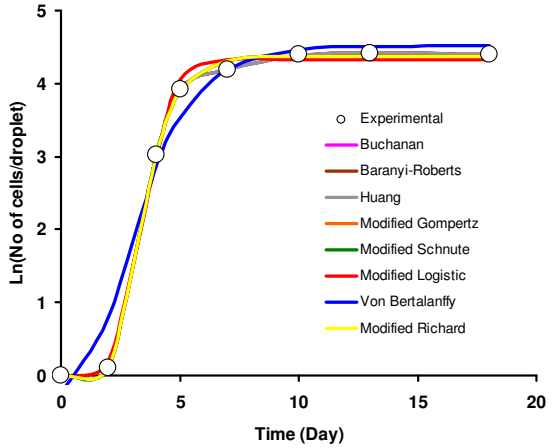


Figure 2. Growth curves of *Chlorella vulgaris* fitted by various growth models available in the literature. The optical density was transformed into natural logarithm.

Table 2. Statistical analysis of the various fitting models.

| Model | p | RMSE | $Ra^2$ | AICc | BF | AF |
|---|---|---|---|---|---|---|
| Buchanan | 3 | 0.00425 | 0.999 | -63.791 | 1.00 | 1.00 |
| Baranyi-Roberts | 4 | 0.00475 | 0.999 | -43.125 | 1.00 | 1.00 |
| Huang | 4 | 0.00475 | 0.999 | -43.125 | 1.00 | 1.00 |
| Modified Gompertz | 3 | 0.06673 | 0.998 | -19.739 | 1.01 | 1.02 |
| Modified Schnute | 4 | 0.07424 | 0.997 | 0.848 | 1.01 | 1.02 |
| Modified Logistic | 3 | 0.11254 | 0.995 | -11.377 | 1.09 | 1.11 |
| Von Bertalanffy | 3 | 0.38741 | 0.944 | 8.401 | 1.29 | 1.35 |
| Modified Richard | 4 | 0.07463 | 0.997 | 0.931 | 1.01 | 1.02 |

Note:

| | |
|---|---|
| p | no of parameters |
| $Ra^2$ | Adjusted Coefficient of determination |
| BF | Bias factor |
| AF | Accuracy factor |

The choice of the Buchanan as the best model is apt since the model is the simplest amongst the eight and it also has three-parameter giving it a higher degrees of freedom, which can be important when a growth curve with a small number of measured points is used. In addition all three parameters have biological meaning due to the highly mechanistic property if the model. The Buchanan three-phase models have been successfully used to

model growth of bacteria bacteria [18–23], algae [24] and worm [25].

Parameters obtained from the growth fitting exercise were maximum specific growth rate ($\mu_{max}$), lag time ($\lambda$) and maximal number of cells achieved per droplet ($Y_{max}$) with the values of 1.301 (day-1), 1.861 (day) and 77 (no of cells/droplet), respectively (Table 3).

Table 3. Fitted growth parameters according to the Buchanan three-phase model.

| Parameters | Fitted values |
|---|---|
| $\mu_{max}$ ($day^{-1}$) | 1.301 |
| Lag time ($\lambda$) (day) | 1.861 |
| $Y_{max}$ (ln no of cells/droplet) | 4.347 |

The parameters determined using the Buchanan model gave a calculated $\mu_{max}$ with a slightly lower than the value reported by Dewan et al. [11] of 1.52 ($day^{-1}$) in the original publication. Other parameters such as lag time, $Y_{max}$ and A were not available from the original publication. The results obtained in this work would be useful in further works such as secondary modeling of the algae for the effects of pH, temperature and substrates on the specific growth rate. There is almost no report on the use of the Buchanan model for fitting algae growth curves in the literature for comparative purposes. The most cited growth model for fitting growth curves of algae is the Gompertz model [26–30].

In conclusion, several of the sigmoidal functions evaluated can be used as primary level microbial growth models with an acceptable degree of goodness-of-fit. However, the best model was the simpler Buchanan three phase model. The parameters obtained from fitting the algae growth curve using this model can bed used for further modeling and optimization exercises for identifying key controlling parameters of the microfluidic devise.

## ACKNOWLEDGEMENT

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