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The Growth of *Paracoccus* sp. SKG on Acetonitrile is Best Modelled using the Buchanan Three Phase Model

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ABSTRACT

Organonitriles are carcinogenic and mutagenic. Bioremediation of acetonitrile, an organonitrile, has been touted as a more economical and feasible method compared to physical and chemical approaches. In this work, we model the growth of growth of *Paracoccus* sp. SKG on acetonitrile from published literature to obtain vital growth constants. These growth constants can only be accurately obtained from mathematical modelling of the growth curves using various available primary models such as logistic, Gompertz, Richards, Schnute, Baranyi-Roberts, Von Bertalanffy, Buchanan three-phase and more recently Huang models. 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). Novel constants obtained from the modelling exercise would be useful for further secondary modelling implicating the effect of media conditions and other factors on the growth of this bacterium on acetonitrile.

INTRODUCTION

Organonitriles are carcinogenic and mutagenic. They are widely used in industry such as the synthesis of plastics, rubber, herbicides, pharmaceuticals, drug intermediates, and pesticides. In addition, acetonitrile, an organonitrile, is extensively utilized in laboratories as a solvent and extractant for HPLC (High Performance Liquid chromatography). Organonitriles are classified as priority pollutants. The global industrial consumption of acetonitrile alone is more than 4×10^4 tonne in 2001 [1,2]. Consequently, wastewaters from the various usages of organonitriles often contain high contents of organonitrile compounds. Bioremediation of acetonitrile has been touted as a more economical and feasible method compared to physical and chemical approaches. Santoshkumar et al [3] has isolated a bacterial strain that could grow on acetonitrile. The growth profile of the strain showed inhibition of growth at elevated concentrations of acetonitrile. Modelling of the growth curves can yield important parameters that could be used for further secondary modelling exercise such as the inhibitory effect of substrate on growth.

The bacterial growth curve can be fitted by various mathematical functions such as Logistic, Gompertz, Richards, Schnute [4], Baranyi-Roberts [5] and Von Bertalanffy [6,7], Buchanan three-phase [8] and more recently the Huang model [9] (**Table 1**). 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.

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	$y=A+y_{max}-\ln\left(e^A+\left(e^{Y_{max}}-e^A\right)e^{-\mu_{max}B(x)}\right)$ $B(x)=x+\frac{1}{\alpha}\ln\frac{1+e^{-\alpha(x-\lambda)}}{1+e^{\alpha\lambda}}$
Buchanan Three-Phase Linear Mode	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 lower asymptote;
n= no of parameters
μ_{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=μ_{max}

The objective of the first part of this work is to evaluate similarities and differences between the models using published available data from [3] that lacks the initial modelling 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 spur further information and improvement in the works already done by researchers.

MATERIALS AND METHOD

Acquisition of Data

In order to process the data, graphs were scanned and electronically processed using WebPlotDigitizer 2.5 [10]. The software helps to digitize scanned plots into table of data with good enough precision [11]. Data were acquired from the works of Santoshkuma et al. [3] from Figure 4 and then replotted.

Fitting of the data

To decide whether there is a statistically substantial difference between models with different number of parameters, in terms of the quality of fit, 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) and corrected AICc (Akaike Information Criterion) [12].

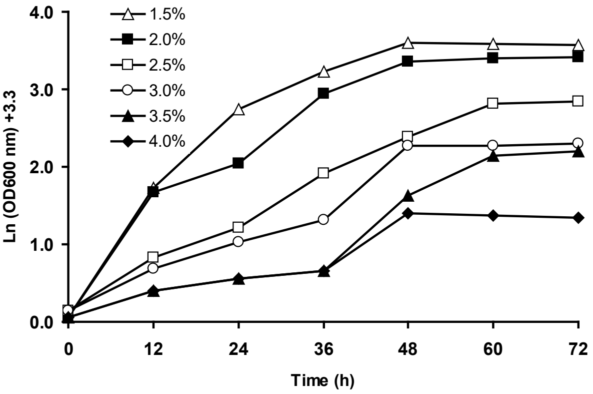

Fig. 1. Refitting of the natural logarithm of growth curve of *Paracoccus* sp. SKG on acetonitrile. The legends depict concentration of acetonitrile (% v/v).

RMSE

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

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

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 (Eqns. **2** and **3**).

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

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

Akaike information criterion with correction (AICc)

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 [13]. AIC deals with the trade-off regarding the goodness of fit of the model along with the intricacy of the model. It is in reality founded on information theory. The procedure offers a comparative approximation of the information lost for every time a certain model is employed to signify the process that produces the information or data. For any output of a collection of predicted models, the most accepted model is the model demonstrating 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 formula includes a number of parameters punishment, the greater the parameters, the less favoured the end result or the greater the AIC value. Therefore, AIC not simply returns goodness of fit, but additionally, doesn't really encourage utilizing more complex model (overfitting) for fitting experimental data. Considering that the data within this work is smaller compared to the number of parameter employed a remedied version of AIC, the Akaike information criterion (AIC) with correction or AICc is employed in its place. The AICc is computed for each and every data set for each model based on the following equation (Eqn. **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 p is the number of parameters of the model and n is the number of data points. The procedure considers the alteration in goodness-of-fit and the improvement in number of parameters between two models. For each and every data set, the model having the smallest AICc value is extremely likely correct [13].

Accuracy Factor (AF) and Bias Factor (BF)

Accuracy Factor (AF) and Bias Factor (BF) to test for the goodness-of-fit of the models as recommended by Ross [14] were also employed. A Bias Factor equal to 1 indicates 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 (Eqns. **5** and **6**).

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

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

RESULTS AND DISCUSSION

Essentially, the most vital results from curve fitting in growth curve model are the capacity to utilize a growth model that have a good fundamental mechanistic function in accordance with good theoretical understanding of the system. Among the finest of such model is the Michaelis-Menten kinetics that models the effects substrate on the initial enzyme activity of an enzyme. To get the best model, eight various growth models were put to use for this study to suit the experimental data. The ensuing fitting illustrates visually sufficient fitting for the models of Huang, modified Gompertz, modified logistics, Von Bertalanffy, Baranyi-Roberts and Buchanan-3-models (**Figs. 2-8**). Other models gave poor fitting and were not shown. The statistical analysis results (**Table 2**) indicated that the Buchanan-three-phase model was the best with highest adjusted R^2, lowest RMSE and AICc values, and Bias and Accuracy Factor values closest to unity. The Buchanan-three-phase model was then used to fit the data and the resultant fitted values obtained (**Table 3**).

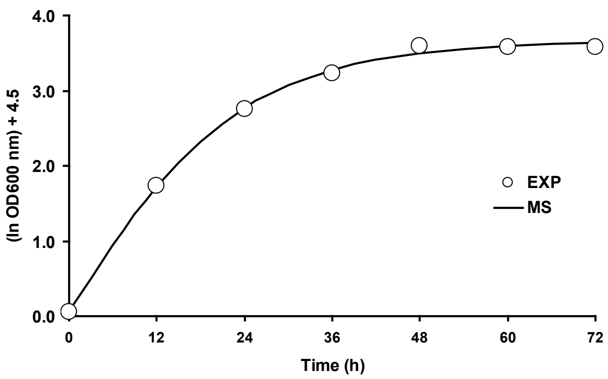

Fig. 2. Growth curves of *Paracoccus* sp. SKG on acetonitrile fitted by the modified Schnute growth model.

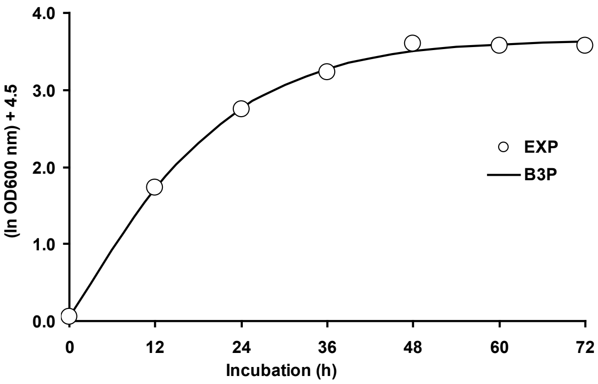

Fig. 3. Growth curves of *Paracoccus* sp. SKG on acetonitrile fitted by the Baranyi-Roberts growth model.

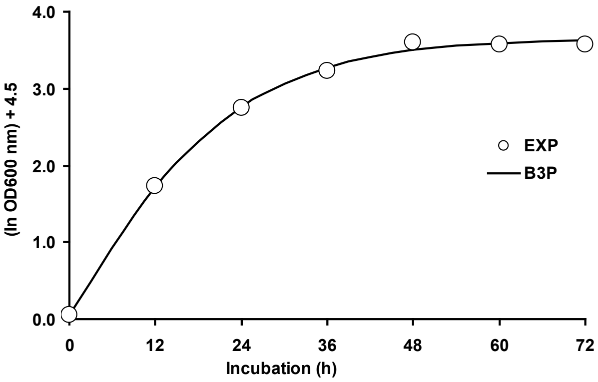

Fig. 4. Growth curves of *Paracoccus* sp. SKG on acetonitrile fitted by the modified Gompertz growth model.

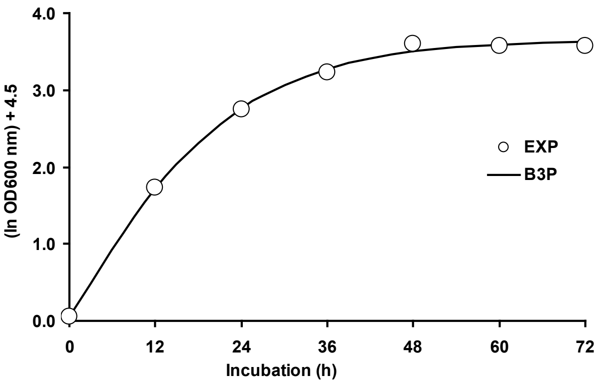

Fig. 5. Growth curves of *Paracoccus* sp. SKG on acetonitrile fitted by the Buchanan-3-phase growth model.

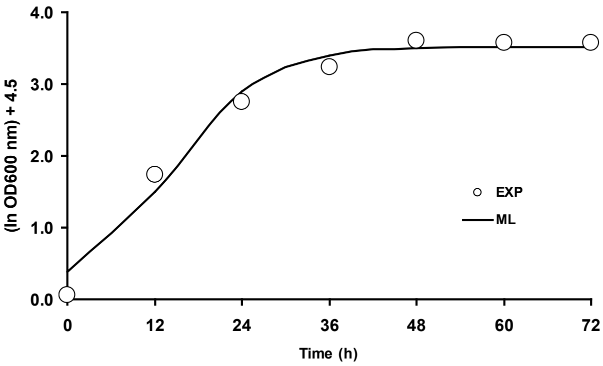

Fig. 6. Growth curves of *Paracoccus* sp. SKG on acetonitrile fitted by the modified logistics growth model.

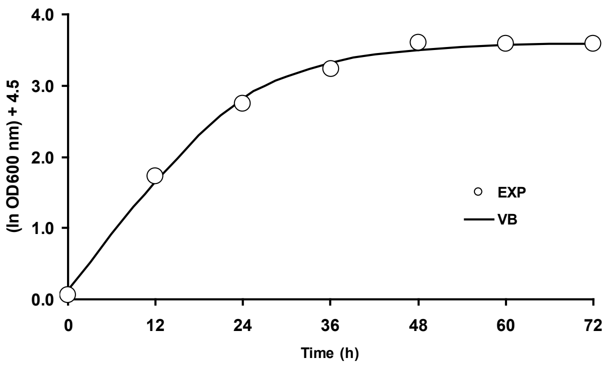

Fig. 7. Growth curves of *Paracoccus* sp. SKG on acetonitrile fitted by the von Bertalanffy growth model.

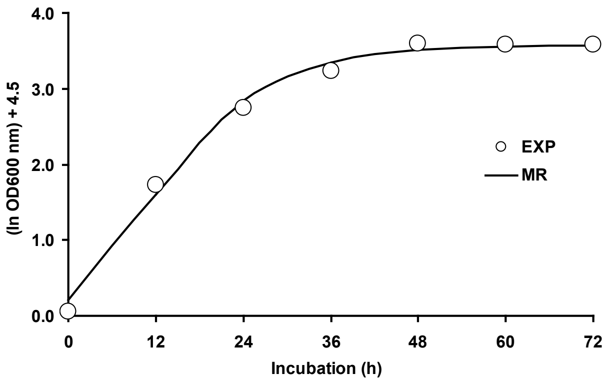

Fig. 8. Growth curves of *Paracoccus* sp. SKG on acetonitrile fitted by the modified Richard growth model.

Table 2. Statistical analysis of the various fitting models.

Model	*n*	RMSE	R^2	adR^2	AF	BF	AICc
Huang (HG)	4	n.a	n.a	n.a	n.a	n.a	n.a
Baranyi-Roberts (BR)	4	0.116	0.996	0.988	1.128	1.097	41.92
Modified Gompertz (MG)	3	0.112	0.995	0.990	1.010	1.169	-0.52
Buchanan-3-Phase (B-3-P)	3	0.060	0.999	0.997	1.010	1.000	-9.31
Modified Richards (MR)	4	0.155	0.993	0.978	1.230	1.190	45.96
Modified Schnute (MS)	4	0.094	0.997	0.992	1.021	1.011	39.02
Modified Logistics (ML)	3	0.235	0.976	0.953	1.359	1.283	9.78
Von Bertalanffy (VB)	3	0.092	0.997	0.993	1.147	1.121	-3.39

Note:

SSE	Sums of Squared Errors
RMSE	Root Mean Squared Error
R^2	Coefficient of Determination
adR^2	Adjusted Coefficient of Determination
AICc	Corrected Akaike Information Criterion
BF	Bias Factor
AF	Accuracy Factor
n	No of parameter
n.a.	Not available

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

Acetonitrile	1.5%	2.0%	2.5%	3.0%	3.5%	4.0%
y0	-0.09	-0.06	-3.4	0.14	0.06	0.06
μ_{max} (h^{-1})	0.09	0.08	0.05	0.04	0.04	0.03
lag (h)	-5.20	-5.20	-76	1.76	9.32	3.40
y_{max}	3.59	3.39	2.83	2.28	2.2	1.36

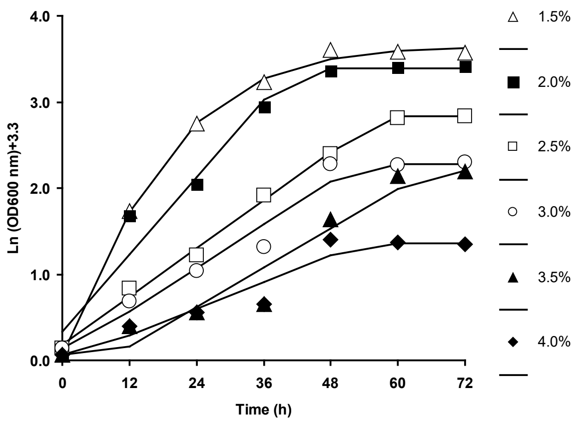

Fig. 9. Fitting of the growth curve of *Paracoccus* sp. SKG on acetonitrile using the Buchanan-three-phase model. The legends depict concentration of acetonitrile (% v/v).

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 [15–18], algae [19] and worm [20].

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 [8,16]. Buchanan et al. [8] 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 $t \le t_{lag}$,
$N_t = N_o$

Exponential growth phase:

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

Stationary phase:

For $t \ge t_{max}$,
$N_t = N_{max}$

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 [8]. Its disadvantage include the fact that it could only fit growth curves having an abrupt transition from the lag phase to exponential phase [21].

Many experts have recommended that whenever a three-parameter model is enough to explain the data, experts recommend over a four-parameter model since three-parameter model is significantly simpler and as a result much easier to use and solution is more stable for the reason that parameters are much less correlated. In addition to 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. Furthermore, it is necessary that all three parameters may be given a biological interpretation.

CONCLUSION

In conclusion, the various models used to fit the growth of *Paracoccus* sp. SKG on acetonitrile as a substrate showed that the best model was Buchanan-three-phase based on statistical analysis. The fitted data from this work can be used in the further optimization works of the microbe.

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