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Modelling the Growth of *Moraxella* sp. B on Monobromoacetic acid (MBA)

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ABSTRACT

Monohalogenated acetic acids have been widely used in industry and agriculture as precursor to chemicals and as herbicides, respectively. Bioremediation of xenobiotics have been touted as a more economical and feasible method compared to physical and chemical approaches. In this work, we model the growth of *Moraxella* sp. B on monobromoacetic acid (MBA) 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). The constants obtained indicated that the lag period was increased as the concentrations of MAB was increased. In addition, the growth rate was severely reduced at high concentrations of MAB indicating substrate inhibition. 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 MAB.

INTRODUCTION

Monohalogenated acetic acids have been widely used in industry and agriculture. Monohalogenated acetic acids include compounds such as monobromoacetic acid (MBA), monofluoroacetic acid (MFA) and monochloroacetic acid (MCAA). As some of these compounds are toxic to plants, they have been used as herbicides [1,2]. In the late 1990s, the United States Environmental Protection Agency USEPA began to regulate the use of HAAs [2] as there were some evidences of human toxicity and carcinogenicity [3]. In addition, some of these compounds are also toxic to aquatic organisms [4], and have been found contaminating surface and underground waters at levels of between parts per trillion to parts per billion [5–7]. This has led to the classification of the entire haloacetic acids as hazardous substances [2].

Monobromoacetic acid (MBA) is also known as bromoethanoic acid, α -Bromoacetic acid, Monobromoacetic acid and carboxymethyl bromide has been used as a chemical intermediate for manufacturing of various chemicals with application in agriculture and pharmacy [8]. Several different bacteria are known to aerobically degrade HAAs either cometabolically [9] or as a sole carbon and energy source [1,2,8,10–13]. The strain *Moraxella* sp. B [14] possesses two haloacid dehalogenases, one active with all monohaloacetates and the other active with MCA and MBA [15]. Torz et al [2] study the growth of this bacterium on monohaloacetates including MBA. The growth curve exhibited sigmoidal properties, but the authors did not capitalize the existence of various primary growth models in order to obtain important growth constants that can be used in further modelling. The

growth profile of the strain showed inhibition of growth at elevated concentrations of MBA. 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 [16], Baranyi-Roberts [17] and Von Bertalanffy [18,19], Buchanan three-phase [20] and more recently the Huang model [21] (Table 1) can be utilized to model bacterial growth curve. Besides exhibiting predictive ability and internal uniformity, which is a must, the potency of a model ought to be looked at by its mathematical straightforwardness, flexibility, the number of its adjustable parameters and, where appropriate, if they have intuitive meaning.

Table 1. Growth models used in this study.

Model	n	Equation
Modified Logistic	3	$y = \frac{A}{1 + \exp\left[\frac{4\mu_{\max}}{A}(\lambda - t) + 2\right]}$
Modified Gompertz	3	$y = A \exp\left\{-\exp\left[\frac{\mu_{\max}}{A}(\lambda - t) + 1\right]\right\}$
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	$y = A + \mu_{\max} x + \frac{1}{\mu_{\max}} \ln\left(e^{-\mu_{\max} x} + e^{-h_0} - e^{-\mu_{\max} x - h_0}\right) - \ln\left(1 + \frac{e^{\mu_{\max} x + \frac{1}{\mu_{\max}} \ln(e^{-\mu_{\max} x} + e^{-h_0} - e^{-\mu_{\max} x - h_0})} - 1}{e^{(y_{\max} - A)}}\right)$
Von Bertalanffy	3	$y = K \left[1 - \left[1 - \left(\frac{A}{K} \right)^3 \right] \exp\left\{ -\left(\frac{t}{\lambda/3K^2} \right)^3 \right\} \right]^{\frac{1}{3}}$
Huang	4	$y = A + y_{\max} - \ln(e^A + (e^{y_{\max}} - e^A) e^{-\mu_{\max} B(x)})$ $B(x) = x + \frac{1}{\alpha} \ln \frac{1 + e^{-\alpha(x-\lambda)}}{1 + e^{-\alpha\lambda}}$
Buchanan Three-Phase Linear Mode	3	$y = A, \text{ if } x < \lambda$ $y = A + k(x - \lambda), \text{ if } \lambda \leq x \leq x_{\max}$ $y = y_{\max}, \text{ if } x \geq x_{\max}$

Note:

A = bacterial lower asymptote;
 n = no of parameters
 $\mu_{\max}$ = 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 $h_0 = \mu_{\max}$

The objective of the first part of this work is to evaluate similarities and differences between the models using published available data from [2] that does not have the initial modelling and also to take care of the issue of which model(s) work extremely well, based on statistical thinking. This could give new data and outcomes, which could encourage additional information and enhancement in the works already performed by researchers.

MATERIALS AND METHOD

Acquisition of Data

In order to process the data, graphs were scanned and electronically processed using WebPlotDigitizer 2.5 [22]. The software helps to digitize scanned plots into table of data with good enough precision [23]. Data were acquired from the works of Torz et al. [2] from Figure 1 and then replotted.

Fitting of the data

To determine whether or not there exists a statistically considerable difference between models with various quantity of parameters, with regards to the quality of fit, data was statistically evaluated by means of numerous methods including the root-mean-square error (RMSE), adjusted coefficient of determination (R^2), bias factor (BF), accuracy factor (AF) and corrected AICc (Akaike Information Criterion) [24].

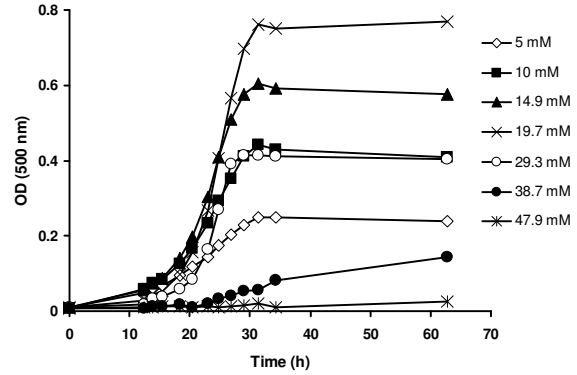


Fig. 1. Refitting of the growth curve of *Moraxella* sp. B on MAB. The legends depict concentrations of MAB.

RMSE

The RMSE was calculated according to Eq. (1), where Pd_i 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).

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

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 (Eqns. 2 and 3).

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

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

Akaike information criterion with correction (AICc)

AIC handles the trade-off concerning the goodness of fit of the model together with the complexity of the model. It is really established on information theory. The Akaike information criterion (AIC) supplies a path for model selection through calculating the comparative quality of a given statistical model for a given number of experimental data [25]. 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 [25].

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 [26] 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 > 1 indicates a fail-safe model. The Accuracy Factor is always ≥ 1 , and higher AF values indicate less precise prediction (Eqns. 5 and 6).

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

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

RESULTS AND DISCUSSION

One of the most important part of the curve fitting exercise is 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 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. The Buchanan-three-phase model was then used to fit the data and the resultant fitted values obtained (Table 3). The constants obtained indicated that the lag period was increased as the concentrations of MAB were increased. In addition, the growth rate was severely reduced at high concentrations of MAB indicating substrate inhibition.

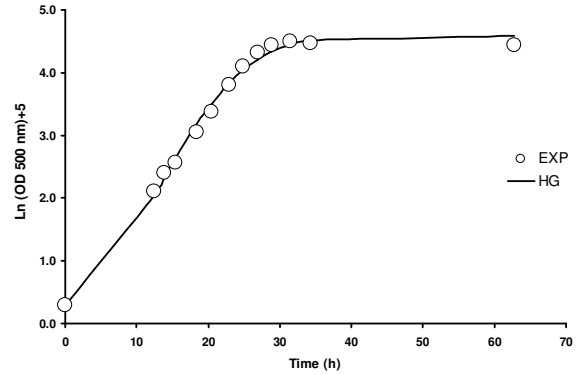


Fig. 2. Growth curves of *Moraxella* sp. B on MAB fitted by the Huang growth model.

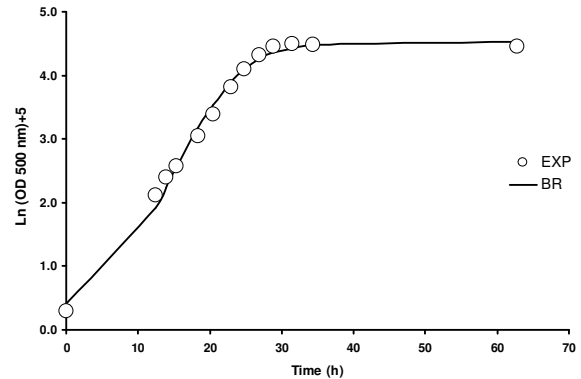


Fig. 3. Growth curves of *Moraxella* sp. B on MAB fitted by the Baranyi-Roberts growth model.

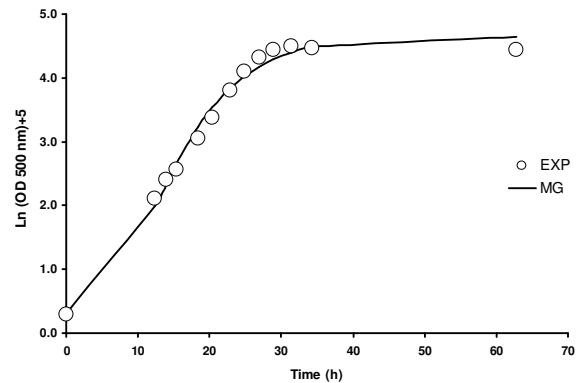


Fig. 4. Growth curves of *Moraxella* sp. B on MAB fitted by the modified Gompertz growth model.

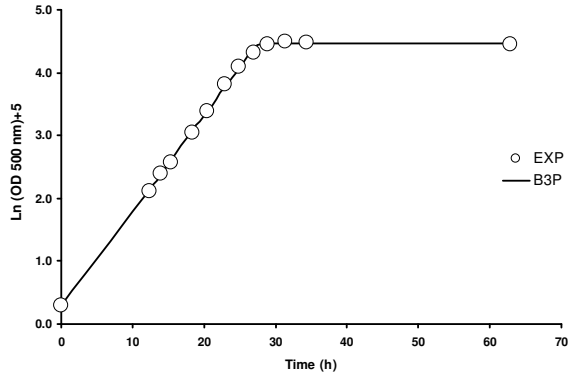


Fig. 5. Growth curves of *Moraxella* sp. B on MAB fitted by the Buchanan-3-phase growth model.

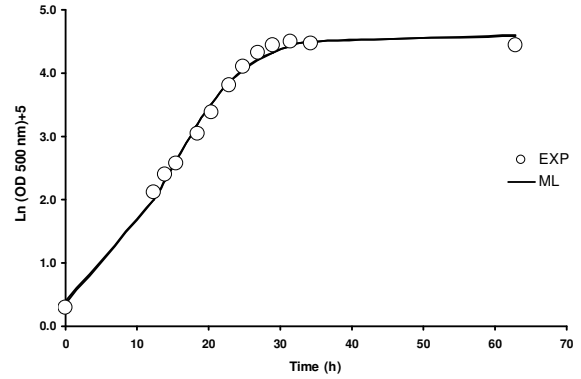


Fig. 8. Growth curves of *Moraxella* sp. B on MAB fitted by the modified logistics growth model.

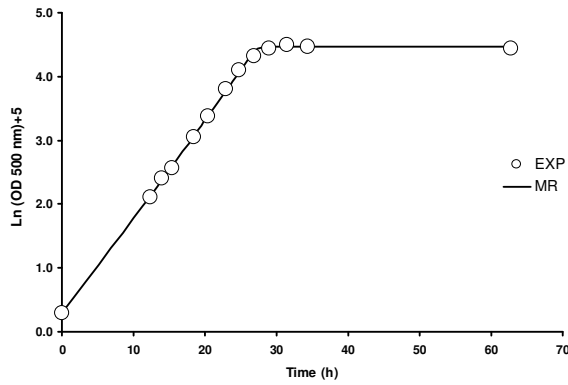


Fig. 6. Growth curves of *Moraxella* sp. B on MAB fitted by the modified Richard growth model.

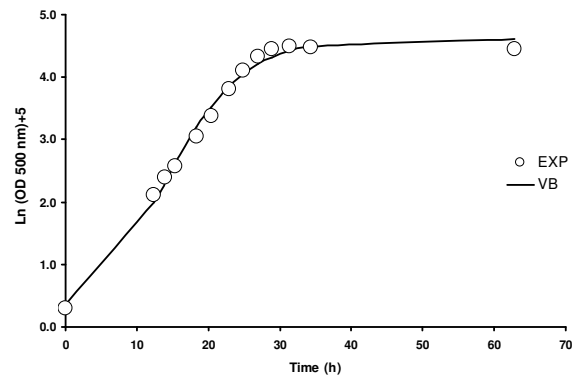


Fig. 9. Growth curves of *Moraxella* sp. B on MAB fitted by the von Bertalanffy growth model.

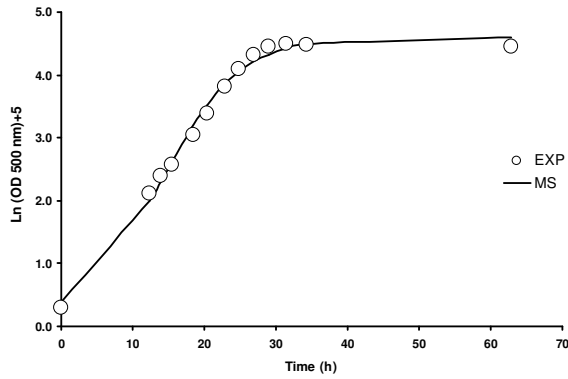


Fig. 7. Growth curves of *Moraxella* sp. B on MAB fitted by the modified Schnute growth model.

Table 2. Statistical analysis of the various fitting models.

Model	p	RMSE	R^2	adR^2	AF	BF	AICc
Huang	4	0.11	0.99	0.99	1.02	1.00	-36.46
Baranyi-Roberts	4	0.13	0.99	0.99	1.05	0.99	-31.86
modified Gompertz	3	0.09	1.00	0.99	1.01	1.00	-47.95
Buchanan-3-phase	3	0.03	1.00	1.00	1.01	1.00	-72.14
modified Richards	4	0.12	0.99	0.99	1.05	1.00	-33.15
modified Schnute	3	0.05	1.00	1.00	1.05	1.00	-55.18
modified Logistics	3	0.12	0.99	0.99	1.04	1.00	-40.51
von Bertalanffy	4	0.18	0.98	0.98	1.03	1.00	-28.88

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

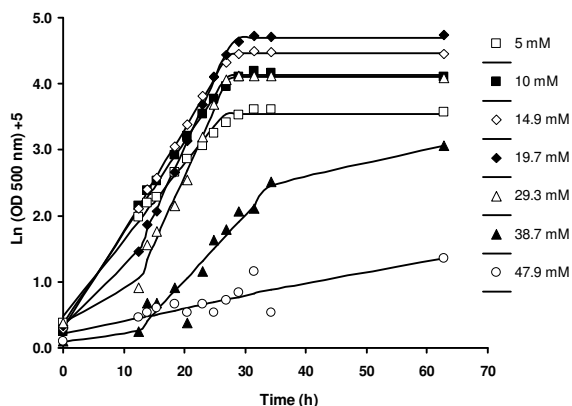


Fig. 10. Fitting of the growth curve of *Moraxella* sp. B on MAB using the Buchanan-three-phase model. The legends depict concentration of MAB (% v/v).

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

	MAB (mM)						
	5	10	14.9	19.7	29.3	38.7	47.9
μ_{max} (h ⁻¹)	0.116	0.139	0.156	0.207	0.206	0.1	0.019
lag (h)	-10.187	-3.389	0.598	6.802	9.125	10.794	12.441
y_{max}	3.545	4.14	4.466	4.705	4.11	3.063	1.359

The B3P model is really a three-parameter model giving it a larger degree of freedom in comparison with four- or five-parameter models. The model certainly is the least difficult among the eight. This is very important every time a growth curve obtaining a smaller number of points is employed. In addition, all three parameters possess biological relevance due to highly mechanistic property of the model. The Buchanan three-phase model has been utilized effectively to model growth of bacteria [27–30], algae [31] and worm [32].

The B3P model is an extension of the Baranyi-Roberts model, as the latter is problematic due to it is rather sensitive to the number and distribution of data points [20,28]. Due to this, Buchanan et al. [20] designed an easier three-phase linear model to solve this issue,

The Buchanan model has several assumptions 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:

$$\text{for } t \leq t_{lag}, \\ N_t = N_o$$

Exponential growth phase:

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

Stationary phase:

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

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)

One of the biggest benefit of the model is its simplicity. Moreover, it produces an approximation in the form of a graphics to the mathematics microbiologists are usually familiar with in calculating the growth kinetic [20]. On of its major drawback is that it could only fit growth curves showing an unexpected conversion from the lag phase to the exponential phase [33].

Many experts have proposed that anytime a three-parameter model is sufficient to describe the data, it's recommended over the four-parameter model due to the fact three-parameter model is substantially simpler and consequently a lot better to utilize and solution is more stable since parameters tend to be less correlated. Additionally, whenever a three-parameter model is utilized, the estimates have more degrees of freedom, and that is crucial any time a growth curve or generation curve with a small number of calculated points is employed. What's more, it is necessary that all three parameters can be given a biological meaning.

CONCLUSION

In conclusion, the various models used to fit the growth of *Moraxella* sp. B on monobromoacetic acid (MAB) as a substrate showed that the best model was Buchanan-three-phase based on statistical analysis. The constants obtained indicated that the lag period was increased as the concentrations of MAB were increased. In addition, the growth rate was severely reduced at high concentrations of MAB indicating substrate inhibition.

The fitted data from this work can be used in the further optimization works of the microbe.

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