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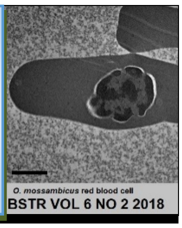

Mathematical Modeling of Molybdenum Blue Production from *Bacillus amyloliquefaciens* strain KIK-12

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ABSTRACT

Molybdenum reduction to molybdenum blue is a detoxification process, and production of Mo-blue is growth associated. Mathematical modelling of the reduction process can reveal important parameters such as specific reduction rate, theoretical maximum reduction and whether reduction at high molybdenum concentration affected the lag period of reduction. The used of linearization method through the use of natural logarithm transformation, although popular, is inaccurate and can only give an approximate value for the sole parameter measured; the specific growth rate. In this work, a variety of models for such as logistic, Gompertz, Richards, Schnute, Baranyi-Roberts, Von Bertalanffy, Buchanan three-phase and more recently Huang were utilized to obtain values for the above parameters or constants from *Bacillus amyloliquefaciens* strain KIK-12. The Mo-blue production from this bacterium was sigmoidal in shape with a lag phase of about 15 hours and reaching maximum Mo-blue production at approximately 50 hours of static incubation. The resultant fitting shows visually acceptable fitting. The best performance was modified Gompertz 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 modified Gompertz model was then utilized to model the Mo-blue production curves to obtain reduction coefficients. The parameter constants successfully developed from this work will be very useful for the development of further secondary models.

INTRODUCTION

Models such as modified Gompertz, modified logistics, Huang, Buchanan–three phase and Baranyi and Roberts are the amongst the most popular primary models as they can accurately model the growth of bacteria under [1]. Despite this, primary modelling is rarely utilized in bacterial growth on xenobiotics or detoxification processes. Secondary modelling exercises deals with the effect of substrate or environmental factors on growth or bacteria metabolic rates. Models such as Haldane, Aiba and Yano are secondary models often used in modelling of substrate inhibition of microbe growing on xenobiotics such as phenol and catechol [2]. The vast majority of models that are widely-used in modelling bacterial growth or metabolite production rates are thought to be either empirical or mechanistic, although in reality most of them lie between these two classifications [1]. Bacterial growth or bacterial-linked processes normally display a unique phase in which the specific growth rate commences at a value of zero after which it accelerates to a maximal value (μ_{max}) in a certain time period, producing a lag time (λ).

Furthermore, growth curves include a final phase where the rate diminishes and ultimately gets to zero, so that an asymptote (A) is achieved. The changes in the growth rate often resulting in a sigmoidal curve, with the characteristics lag phase just after t = 0. This is followed by an exponential phase, a stationary phase and finally the death phase [3]. Aside from the asymptotic value and the lag period, another valuable parameter of the growth curve is the maximum specific growth rate (μ_m). This value is often used in the development of secondary models such as the effects of substrate, product, pH and temperature on growth rate of organism.

Widespread use of molybdenum in industry such as an alloying agent, automobile engine anti-freeze component, portion of corrosion resistant steel and also as lubricant in the form of molybdenum disulphide has caused several water pollution situations worldwide such as in the Tokyo Bay, Tyrol in Austria and in the Black Sea, where molybdenum level actually gets to in the hundreds of ppm [4]. Molybdenum is very toxic to ruminants at levels of several parts per million, with cows being the most affected [5,6]. It is also a genotoxic agent [7].

Table 1. Mo-blue production 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	$y=A\left\{1+v\exp(1+v)\exp\left[\frac{\mu_m}{A}(1+v)\left(1+\frac{1}{v}\right)(\lambda-t)\right]\right\}$
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	$y=A+\mu_m x+\frac{1}{\mu_m}\ln\left(e^{-\mu_m x}+e^{-h_0}-e^{-\mu_m}\right.$ $-\ln\left(1+\frac{e^{\mu_m x+\frac{1}{\mu_m}\ln\left(e^{-\mu_m x}+e^{-h_0}-e^{-\mu_m x-h_0}\right)}-1}{e^{(y_{\max}-A)}}\right)$
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	$y=A+y_{\max}-\ln\left(e^A+\left(e^{Y_{\max}}-e^A\right)e^-\right.$ $B(x)=x+\frac{1}{\alpha}\ln\frac{1+e^{-\alpha(x-\lambda)}}{1+e^{\alpha\lambda}}$
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= Mo-blue lower asymptote;
μ_m= maximum specific Mo-blue production rate;
v= affects near which asymptote maximum Mo-blue production occurs.
λ=lag time
y_{max}= Mo-blue 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 reduction process. The lag time (h^{-1}) can be calculated as h_0=μ_{max}

More recent data on molybdenum toxicity have shown that it inhibits spermatogenesis and arrests embryogenesis in organisms such as catfish and mice at levels as low as several parts per million [8,9] As to date quite a number of Mo-reducing bacteria have been isolated, and most of these bacteria were isolated locally [10–16,16,17] with the exception of a few [18–21]. Kinetic studies on Mo-blue production have been explored previously [22,23] but all of these works utilize the linearization of the Mo-blue production over time profile to obtain the specific growth rate for further secondary modelling. As the benefits of nonlinear regression analysis of the Mo-blue production have been described above, thus, the objective of this work is to evaluate several available models such as Logistic [3,24], Gompertz [3,25], Richards [3,26], Schnute [3], Baranyi-Roberts [27], Von Bertalanffy [28,29], Buchanan three-phase [30] and more recently Huang model [1] (**Table 1**) in modeling Mo-blue production from the bacterium

MATERIALS AND METHODS

Growth and maintenance of *Bacillus amyloliquefaciens* strain KIK-12

The bacterium was previously isolated, identified and characterized by Yakasai et al. [31]. The growth and maintenance was carried out on solid agar in low phosphate media agar compose of Na_2HPO_4 (0.071% or 5 mM), $Na_2MoO_4.2H_2O$ (0.242 % or 10 mM), NaCl (0.5%), $(NH_4)_2.SO_4$ (0.3%), $MgSO_4.7H_2O$ (0.05%), yeast extract (0.5%) and glucose (1%) at pH 7.0 and incubated for 48 hours at room temperature. Resting cells for molybdenum reduction studies was prepared as before [31]. The production of Mo-blue from the media in a microplate format was measured using the specific extinction coefficient of 11.69 $mM.^{-1}.cm^{-1}$ at 750 nm as the maximum filter wavelength available for the microplate unit was 750 nm.

Determination of Kinetic Parameters for Molybdenum Blue production

Fitting of the data

Fitting of the growth data utilizes the CurveExpert Professional software (Version 2.6) that relies on the Marquardt algorithm that minimizes sums of square of residuals. The steepest ascent search was utilized to estimate the μ_m, while intersection of this line to the x axis is λ. The final datum point is the asymptote (A). The Huang's model was solved numerically using an ode45 solver in MATLAB (Version 7.10.0.499, The MathWorks, Inc., Natick, MA).

Statistical analysis

Statistical significant difference determination between the models having various number of parameters was evaluated through numerous methods such as the corrected AICc (Akaike Information Criterion), Root-Mean-Square Error (RMSE), bias factor (BF), accuracy factor (AF), and adjusted coefficient of determination (R^2) as before.

RESULTS AND DISCUSSION

The Mo-blue production from this bacterium was sigmoidal in shape with a lag phase of about 15 hours and reaching maximum Mo-blue production at approximately 50 hours of static incubation (**Fig. 1**). The Mo-blue production over time profile was fitted to eight different models. The resultant fitting shows visually acceptable fitting (**Fig. 2**). The best performance was modified Gompertz 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 Von Bertalanffy with the lowest score for most of the statistics tests (**Table 2**). The modified Gompertz model was then utilized to model the Mo-blue production curves (**Fig. 3**). The coefficients for the modified Gompertz model at various molybdenum concentrations are shown in **Table 3**.

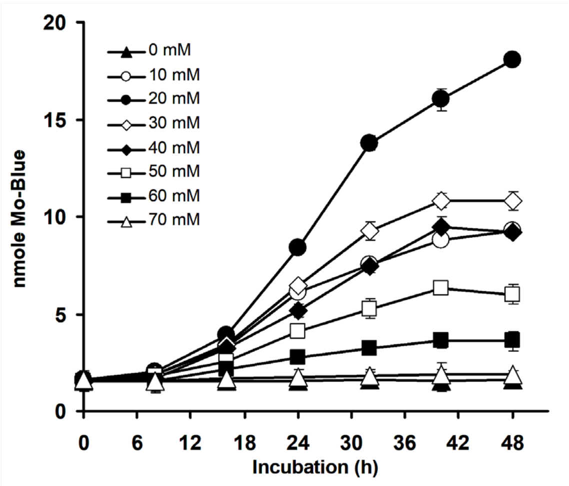

Fig. 1. The Mo-blue production curves of *Bacillus amyloliquefaciens* strain KIK-12 measure at various molybdenum (sodium molybdate) with respect to time. The error bars represent mean ± standard deviation of three replicates.

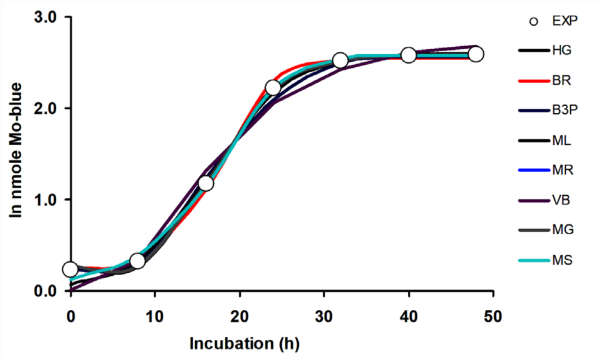

Fig. 2. The Mo-blue production curve of *Bacillus amyloliquefaciens* strain KIK-12 at 40 mM of sodium molybdate fitted to various models. The models utilized were Huang (HG), Baranyi-Roberts (BR), Buchanan-three phase (B3P), modified Logistics (ML), modified Richards (MR), von Bertalanffy (VB), modified Gompertz (MG) and modified Schnute (MS).

Table 2. Statistical analysis of the various fitted models.

Model	p	RMSE	adR^2	AICc	BF	AF
Huang	4	0.048	0.999	-64.15	1.11	1.24
Baranyi-Roberts	4	0.128	0.991	-36.66	1.20	1.27
Buchanan-3-phase	3	0.020	0.993	-94.76	1.01	1.09
modified Logistics	3	0.089	0.996	-52.55	0.99	1.20
modified Richards	4	0.022	0.990	-86.42	0.82	1.24
von Bertalanffy	3	0.101	0.994	-49.04	0.58	1.88
modified Gompertz	3	0.022	0.999	-99.79	1.01	1.01
modified Schnute	4	0.089	0.995	-47.00	1.24	1.26

Note:
p no of parameters
adR^2 Adjusted Coefficient of determination
BF Bias factor
AF Accuracy factor

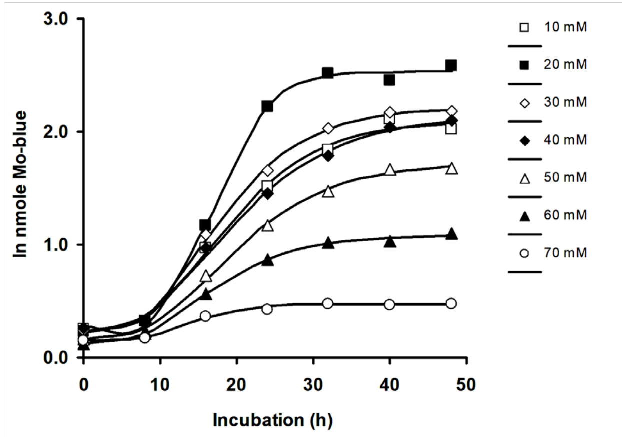

Fig. 3. The Mo-blue production curves of *Bacillus amyloliquefaciens* strain KIK-12 on various concentrations of sodium molybdate fitted using the Gompertz model.

Table 3. Mo-blue production coefficients at various molybdenum concentrations as modelled using the modified Gompertz model.

	Molybdenum concentration										
	5 mM	10 mM	15 mM	20 mM	25 mM	30 mM	35 mM	40 mM	50 mM	60 mM	70 mM
Asymptote (ln nmole Mo-blue)	0.45	1.29	1.80	2.59	3.54	3.69	3.14	1.71	1.47	0.86	0.50
μ_m (h^{-1})	0.02	0.06	0.10	0.12	0.14	0.15	0.14	0.13	0.08	0.06	0.04
lag (h)	6.92	11.37	12.36	12.13	12.07	12.45	12.89	13.86	13.56	13.44	15.07

The modified Gompertz model is one of the classical growth models that include model such as the Verhulst [3,25]. The Gompertz function, named in 1844-1845 by Pierre François Verhulstis, is based on an exponential relationship between specific growth rate and population density. The initial stage of growth is approximately exponential; then, as saturation begins, the growth slows, and at maturity, growth stops. Gibson et al. [32] were the first to use the Gompertz equation to fit microbial growth curves and the equation was successfully used to describe the exponential and stationary phases of the microbial growth curves that is sigmoidal. However, the model was not adequate to describe the lag phase. The model was modified by Gibson et al. [32] to incorporate the lag phase, and have been successfully used in modelling many microbial growth curves to the point where its dominance in mathematically modelling bacterial growth and product formation curves have been acknowledged [3,29,33].

The modified Gompertz model has been extensively used to model the growth of bacteria and bacterial secondary products production such as biohydrogen, methane, lactic acid, biofuel and bacterioricin to name a few [34–38] including Mo-blue production in various bacteria [39–41]. In addition these models have not been applied in the modelling metal microbial detoxification studies although secondary modelling studies have used the Haldane [22,42–44] and several Mo-reducing bacteria (**Table 4**).

Table 4. Primary and secondary models utilized of molybdenum reduction in bacteria.

Mo-reducing bacterium	Best primary model	Best secondary model	Ref
S. marcescens strain Dr.Y6	Modified Gompertz	n.a.	[40]
Burkholderia sp. strain Neni-11	Modified Gompertz	n.a.	[39]
Serratia marcescens strain DR.Y10	Modified Gompertz	n.a.	[45]
Pseudomonas sp. strain DRYJ7	Modified Gompertz	n.a.	[46]
Serratia sp. strain HMY1	Modified Gompertz	n.a.	[41]
Pseudomonas sp. strain DRY1	Modified Gompertz	Wang, modified Han-Levenspiel and Liu (metal)	[47]
Bacillus sp. Strain A.rzi	n.a.	Luong	[22]
Bacillus pumilus strain Lbna	n.a.	Luong	[23]
Serratia sp. strain MIE2	n.a.	Luong	[48]

Parameters obtained from the fitting exercise would be later used for secondary modelling of Mo-blue production using model such as the two-parameter Monod model or other more complex models "secondary models" such as Haldane, Aiba, Yano and others. These mechanistic models are used in basic research and are aimed to reach a better understanding of the physical, chemical and biological processes that lead to the growth profile seen. All other things being equal, mechanistic models are more powerful since they tell you about the underlying processes driving patterns. They are more likely to work correctly when extrapolating beyond the observed conditions [49].

CONCLUSION

In conclusion, the Gompertz model was the best model in modelling the Mo-blue production curve from the Mo-redducing bacterium *Bacillus amyloliquefaciens* strain KIK-12 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 usage of bacterial growth models to acquired precise Mo-blue production rate is helpful for additional secondary model development for molybdenum reduction to Mo-blue particularly and in heavy metals detoxification process generally speaking as evaluated from literature search, and this work has revealed the usefulness of such models. Existing works consist of secondary modelling of the Mo-blue production from this bacterium particularly on the inhibitory effect of the substrate molybdenum on the maximum Mo-blue production rate values extracted from this works. Furthermore, other secondary modelling works such as the effect of environmental conditions (pH and temperature) on Mo-blue production rates are being accomplished.

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