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Mathematical Modelling of Molybdenum Reduction to Mo-blue by a Cyanide-degrading Bacterium

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ABSTRACT

Molybdenum, an emerging pollutant, has being demonstrated recently to be toxic to spermatogenesis in several animal model systems. Metal mines especially gold mine often use cyanide and hence isolation of metal-reducing and cyanide-degrading bacteria can be useful for the bioremediation of these pollutants. Preliminary screening shows that three cyanide-degrading bacteria were able to reduce molybdenum to molybdenum blue (Mo-blue) when grown on a molybdate low phosphate minimal salts media. Phylogenetic analyses of the 16S rRNA gene of the best reducer indicates that it belongs to the *Serratia* genus. A variety of mathematical models such as logistic, Gompertz, Richards, Schnute, Baranyi-Roberts, von Bertalanffy, Buchanan three-phase and Huang were used to model molybdenum reduction, and the best model based on statistical analysis was modified Gompertz with lowest values for RMSE and AICc, highest adjusted R^2 values, with Bias Factor and Accuracy Factor nearest to unity (1.0). The reduction constants obtained from the model will be used to carry out secondary modelling to study the effect of various parameters such as substrate, pH and temperature to molybdenum reduction.

INTRODUCTION

Bacterial growth linked processes frequently 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). It has been argued that the sigmoid shape seen in the lag period is because the bacterial cells are gearing their growth mechanism to adjust to a new environment in a vegetative state especially during storage. This adjustment period is traditionally called the lag period. It has been suggested as a transient period that connects two autonomous systems. The introduction of the lag time or parameter is meant largely convenience rather than having a mechanistic interpretation [1]. It is theorized that in the initial inocula, each bacterial cells would have different rates of growth and if these rates could be measured, would show nonlinear distribution as suggested by several workers [1,2].

Molybdenum has many uses in industries including an alloying agent, automobile engine anti-freeze component, a portion of corrosion resistant steel and as a lubricant in the form of molybdenum disulphide. The wide application of molybdenum in the industry has triggered several water pollution cases worldwide such as in Tokyo Bay, Tyrol in Austria and in the Black Sea, where molybdenum levels reached hundreds of ppm [3]. In addition, terrestrially, it has been recognized as a significant pollutant in sewage sludge pollution that poses a health hazard [3].

Molybdenum is very toxic to ruminants at several parts per million levels, with cows being the most affected [4,5]. A

number of Mo-reducing bacteria had been isolated to date, most of which were locally isolated [6–14] except a few [15–18]. The perceived low toxicity of molybdenum to human and other organism compared to other heavy metals such as mercury, selenium and chromium has resulted in limited works on molybdenum bioreduction as a detoxification process. However, more recent data on molybdenum toxicity in spermatogenesis inhibition and arresting embryogenesis in organisms such as catfish and mice at levels as low as several parts per million [19,20] will spur more works on microbial molybdenum detoxification in the near future.

Kinetic studies on Mo-blue production have been explored previously [8,21], but all these works utilize the linearization of the Mo-blue production over time profile to obtain the specific growth rate for further secondary modelling. As benefit of nonlinear regression analysis for Mo-blue production have been described, thus, the objective of this work is to evaluate several available models such as Logistic [22,23], Gompertz [23,24], Richards [23,25], Schnute [23], Baranyi-Roberts [26], von Bertalanffy [27,28], Buchanan three-phase [29] and more recently Huang model [30] in modeling Mo-blue production from the bacterium *Serratia* sp. strain HMY1.

MATERIAL AND METHODS

Isolation and maintenance of the Molybdate-reducing bacterium

The bacterium utilized in this work has been tentatively identified as *Serratia* sp. strain HMY1 (Yakasai et al., unpublished results). The growth and maintenance were carried out on solid agar in low phosphate molybdate media, LPM (pH 7.0) containing glucose (1%), $(NH_4)_2SO_4$ (0.3%), $MgSO_4.7H_2O$ (0.05%), NaCl (0.5%), yeast extract (0.05%), $Na_2MoO_4.2H_2O$ (0.242%) and Na_2HPO_4 (0.071% or 5 mM) (Abo-Shakeer et al., 2013). Glucose was separately autoclaved. The only difference between the LPM and high phosphate molybdate medium (HPM) was the phosphate concentration, which was fixed at 100 mM for the HPM.

Preparation of resting cells for molybdenum reduction characterization

Mo-blue production was monitored at various molybdate concentrations using resting cells under static conditions in a microplate or microtiter format [31]. Cells were grown in High Phosphate media, HPM (1 L) for 48 h at room temperature on an orbital shaker (150 rpm). The cells were then harvested by centrifuging at 15,000 ×g for 10 min at room temperature. The pellet was washed twice with deionized water to remove residual phosphate. The pellet was re-suspended in LPM (20 mL) minus the molybdenum component to get an approximate absorbance of 1.0 at 600. Resting cells (180 µL) were sterically pipetted into the wells of a sterile microplate.

To initiate production of Mo-blue, various sodium molybdate concentrations (20 µL) from a stock solution were mixed to the resting cells. The plate was incubated at room temperature, following sealing with a tape that allows gas exchange (Corning® microplate). Measurement of Mo-blue production was performed at 750 nm on a BioRad 680 reader (Richmond, CA, USA). A specific extinction coefficient of 11.69 $mM.^{-1}.cm^{-1}$ at 750 nm was utilized [32].

Determination of Kinetic Parameters for Molybdenum Blue production

Fitting of the data

Fitting of the growth data to the nonlinear equations (**Table** 1) was carried out by nonlinear regression utilizing the Marquardt algorithm that minimizes sums of the square of residuals utilizing CurveExpert Professional software (Version 1.6). In this lookup approach, the sum of squares of the differences between the predicted and observed values is minimized. The software can be automatically or manually programmed to calculate initial values of parameters. Estimation of μ_m was carried out by the steepest ascent search of the curve amongst four datum points.

Table 1. Mo-blue production models used in this study.

Model	No of parameters	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	$y = A\left\{1 + v\exp(1+v)\exp\left[\frac{\mu_m}{A}(1+v)\left(1+\frac{1}{v}\right)(\lambda - t)\right]\right\}^{\left(\frac{-1}{v}\right)}$
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	$y = A + \mu_m x + \frac{1}{\mu_m}\ln\left(e^{-\mu_m x} + e^{-h_0} - e^{-\mu_m x - h_0}\right) - \ln\left(1 + \frac{e^{\mu_m x + \frac{1}{\mu_m}\ln\left(e^{-\mu_m x} + e^{-h_0} - e^{-\mu_m x - h_0}\right)} - 1}{e^{(y_{max} - A)}}\right)$
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	$y = A + y_{max} - \ln\left(e^A + \left(e^{Y_{max}} - e^A\right)e^{-\mu_m B(x)}\right)$, $B(x) = x + \frac{1}{\alpha}\ln\frac{1 + e^{-\alpha(x-\lambda)}}{1 + e^{\alpha\lambda}}$
Buchanan Three-phase linear model	3	Y = A, IF X < LAG Y=A + K(X–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 οχχυρσ.
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 $h_0=\mu_{max}$

Estimation of λ was carried out by determining the intersection of this line with the x-axis. Finally, estimation for

the asymptote (A) was carried out by taking the final datum point. As the Huang's model is a differential equation, it needs to be solved numerically. The Runge-Kutta method was utilized to solve numerically the differential equation. The ode45 solver in MATLAB (Version 7.10.0499, The MathWorks, Inc., Natick, MA) was used to solve this equation.

Statistical analysis

The quality of fit of the models to the experimental data was evaluated statistically using the adjusted coefficient of determination (R^2) (**Eqn. 1**), Root-Mean-Square Error (RMSE) (**Eqn. 2**), corrected AICc (Akaike Information Criterion) (**Eqn. 3**), bias factor (BF) (**Eqn. 4**) and accuracy factor (AF) (**Eqn. 5**).

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

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

$$AICc=2p+n\ln\left(\frac{RSS}{n}\right)+2(p+1)+\frac{2(p+1)(p+2)}{n-p-2} \quad \textbf{(Eqn. 3)}$$

Where n represents the number of data points in the curve and p represents the number of parameters used in the model. The model having the smallest AICc value is more likely correct [33].

$$\text{Bias factor} = 10^{\left(\sum_{i=1}^{n}\log\frac{(Pd_i/Ob_i)}{n}\right)} \quad \textbf{(Eqn. 4)}$$

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

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 closest to 1.0. Accuracy Factor (AF) and Bias Factor (BF) were suggested by Ross [34].

A Bias Factor that is equal to 1 signifies an ideal match between observed and predicted values. For microbial growth curves or Mo-blue production studies, a bias factor having values < 1 signifies a fail-dangerous model while a bias factor having value > 1 signifies a model that is fail-safe. For the Accuracy Factor, values are normally ≥ 1, and higher AF values indicates a model which is less precise. The poorest performance was von Bertalanffy with the lowest score for most of the statistics tests (**Table** 2). The coefficients for the modified Gompertz model at various molybdenum concentrations are shown in **Table 3**.

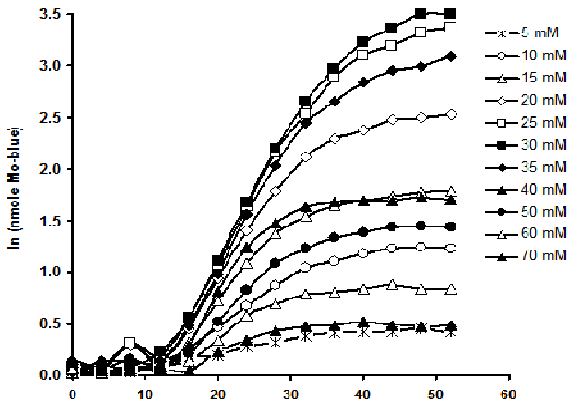

Fig. 1. The Mo-blue production curves of *Serratia* sp. strain HMY1 at various concentrations of sodium molybdate over time. The error bars represent the mean ± standard deviation of three replicates.

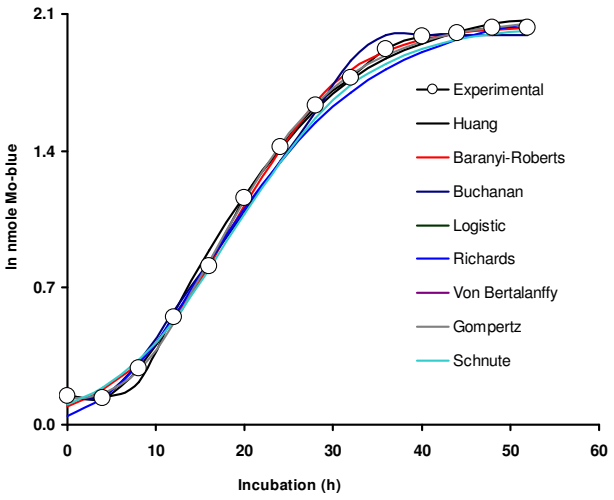

Fig. 2. The Mo-blue production curve of *Serratia* sp. strain HMY1 at 30 mM of sodium molybdate fitted to various models.

Table 2: Statistical analysis of the various fitted models.

Model	RMSE	R^2	adR^2	AF	BF	AICc
Huang	0.0431	0.9975	0.9963	1.05	0.99	-67.28
Baranyi-Roberts	0.0321	0.9986	0.9980	1.07	0.99	-75.52
Modified Gompertz	0.0256	0.9990	0.9987	1.04	1.00	-87.57
Buchanan-3-phase	0.0508	0.9961	0.9949	1.04	1.00	-68.35
modified Richards	0.0720	0.9927	0.9894	1.13	0.90	-52.90
modified Schnute	0.0523	0.9960	0.9942	1.09	0.98	-61.85
modified Logistics	0.0586	0.9944	0.9927	1.09	1.00	-64.35
von Bertalanffy	0.0814	0.9899	0.9869	1.35	0.76	-55.17

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

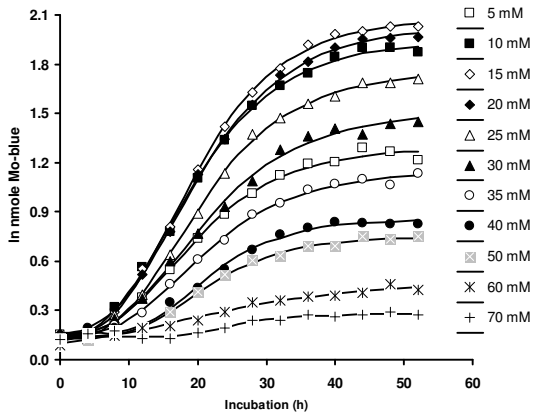

Fig. 3. The Mo-blue production curves of *Serratia* sp. strain HMY1 at various concentrations of sodium molybdate fitted using modified Gompertz model.

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

Conc. (mM)	5	10	15	20	25	30	35	40	50	60	70
Y_0	0.137	0.104	0.127	0.151	0.119	0.129	0.146	0.156	0.134	0.087	0.142
Lag	7.401	6.261	7.335	7.426	7.965	7.448	8.58	11.68	10.37	2.118	18.76
Y_{max}	1.289	1.946	2.085	2.024	1.75	1.503	1.138	0.855	0.752	0.476	0.278
μ_{amax}	0.048	0.074	0.082	0.076	0.066	0.052	0.041	0.035	0.028	0.019	0.014

The modified Gompertz model named in 1844-1845 by Pierre François Verhulst, is a classical growth models that encompasses model such as the Verhulst [24,35]. The first phase of growth is roughly rapid; after that, as saturation commences, the growth decreases, and at maturation, growth ceases. The first person to utilize the Gompertz formula to suit microbial growth curves was Gibson et al. [36], and the equation was used successfully to explain the exponential and stationary stages 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. [44] to incorporate the lag phase and have been successfully used in modelling many microbial growth curves to the point where its dominance in mathematical modelling bacterial growth and product formation curves have been acknowledged [21,26].

The model has its drawbacks and is not perfect with several main issues. Firstly, in the static version, $N_{(t=0)}$ is not equal to N_o. Secondly, an inflexion point is the intrinsic property of the sigmoidal curve causing the model to have a systematic problem in describing the exponential phase [24]. Finally, the model tends to over-estimates its parameter values [37–39].

The asymmetrical sigmoidal shape of the modified Gompertz represents and may offer greater flexibility than the logistic. Sigmoidal models such as the logistic and Gompertz differ chiefly at the point of inflexion between the lower and the upper asymptotes with the logistics and Gompertz models having the distance of 1/2 and 1/e between the lower and the upper asymptotes, respectively [28]. In essence, other growth models provide flexible slope function and variable point of inflexion between the lower and upper asymptotes. These functions are either special or simpler cases of a parent growth model. For instance, the Richard model incorporates the logistics, Gompertz or von Bertalanffy growth models [23,28,36].

Parameters obtained from the fitting exercise would later be used for secondary modelling of Mo-blue production using a model such as the two-parameter Monod model or other more complex "secondary models" such as Haldane, Aiba, Yano and others.

CONCLUSION

In conclusion, the modified Gompertz model was the best model in modelling the Mo-blue production curve of *Serratia* sp. strain HMY1 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 use of bacterial growth models to obtained accurate Mo-blue production rate is useful for further secondary model development in molybdenum reduction to Mo-blue specifically and in heavy metals detoxification process in general as judged from the literature search, and this work has demonstrated the applicability of such models. Current works include secondary modelling of the Mo-blue production from this bacterium especially on the inhibitory effect of the substrate molybdenum on the maximum Mo-blue production rate values obtained from this works. In addition, other secondary modelling works including the effect of environmental conditions (pH and temperature) on Mo-blue production rates are being carried out.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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