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Mathematical Modeling of Molybdenum Blue Production from *Bacillus* sp. Strain Khayat

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ABSTRACT

In the long run, bioremediation is the utmost cost-effective way, particularly at low concentrations while other methods like physical or chemical procedures would be ineffective, for the elimination of heavy metals and organic pollutants. The process of reducing molybdenum (sodium molybdate) with an oxidation state of (VI) to molybdenum blue (oxidation state from V to VI) serves as a form of detoxification. Important characteristics, such as specific reduction rate, theoretical reduction maximum, and the lag duration of reduction, can be shown by mathematical modeling of the reduction process. While natural logarithm transformation is a common linearization approach, it is not precise and can only provide a rough estimate of the most important single measurable parameter; the specific growth rate. In this study, for the first time, values for the aforementioned parameters or constants were calculated using a wide range of models, including the logistic, Gompertz, Richards, Schnute, Baranyi-Roberts, Buchanan three-phase, von Bertalanffy and most recently, the Huang model. Based on statistical tests including root-mean-square error (*RMSE*), bias factor (*BF*), adjusted coefficient of determination ($adjR^2$), accuracy factor (*AF*), and corrected Akaike information criterion (*AICc*), the logistics model was found to be the best model for representing the Mo-blue production curve of *Bacillus* sp. strain khayat. The fitting technique resulted in the calculation of three parameters: specific reduction rate (h^{-1}), Lag period (*h*), and maximum Mo-blue production (nmole Mo-blue). In this study, we utilize a mathematical technique to determine the reduction parameters for Mo-blue production from sodium molybdate. The calculated parameter constants will be used to create secondary models, such as the influence of substrate and environment on Mo-blue synthesis.

INTRODUCTION

Mathematical modeling of bacterial growth and the production of bacterial-related products is possible by employing empirical or mechanistic models such as the logistics model, the Gompertz model, the Baranyi-Roberts model, and others. In addition, each of these models is ranked as either primary, secondary, or tertiary, depending on its level of importance. The majority of models center their attention on the mathematical expressions that serve as the basis for important microbiological phenomena like as proliferation, inactivation, and persistence. Quantification of these activities can be accomplished by the use of cell forming units per milliliter, optical density, dry and wet weights, and other methods along the same lines [1,2]. Following models study how changes to primary model settings (such as temperature, water activity, pH, etc.) have an effect on important variables. The combination of the primary and secondary models that are included in a set of predictive software is referred to as the tertiary model [3]. Primary modeling of microbial growth or product generation, which may include metal detoxification processes, may provide some of the most important information

for secondary modeling. This information may be gathered via primary modeling.

The pervasive use of molybdenum in activities such as in industries producing alloys, vehicle engine anti-freeze components, corrosion-resistant steel, and molybdenum disulphide as a lubricant has been linked to a number of cases of water contamination. These cases include those in which molybdenum was used as a lubricant. Although it is one of the necessary heavy metals that are only required in extremely minute quantities, molybdenum poses a threat to many different kinds of organisms when it is present in higher concentrations. It has potential use in a wide variety of commercial and manufacturing contexts. In the industrial sector, the use of molybdenum in significant quantities has led to severe problems with water contamination on a global scale. It was found that a uranium mill in the southern part of the state of Colorado in the United States was discharging tailings water that contained dissolved Mo values of up to 900 mg/L.

Concentrations of dissolved molybdenum in the aqueous discharge of large molybdenum mills in Colorado can reach up to about 25 mg/L [4], while concentrations of dissolved molybdenum in the aqueous discharge of large open pit copper mines in Arizona range from approximately 1 mg/L to about 30 mg/L [5, 6]. Molybdate is a pollutant that has been found in soil and water at quantities of up to approximately 2000 parts per million (20.8 Mm) [5]. Because of the neighboring Alaverdi copper molybdenum mine, about 300 square kilometers of soil in Armenia has been tainted with heavy metals such as lead, copper, and molybdenum. The concentrations of heavy metals can be up to 40 times higher than the permissible limit in areas that are close to mines [6]. This results in severe pollution. In parts of the Black Sea, the concentration of molybdenum has been measured to be in the hundreds of parts per billion (ppb) [7].

Molybdenum is extremely poisonous to ruminants at concentrations as low as a few parts per million; cows are especially susceptible to its effects [8,9]. There have been quite a few Mo-reducing bacteria found up to this point, the most majority of which have been isolated locally [10-17,18], with a few notable outliers [19-22]. In spite of the fact that it is a heavy metal, molybdenum is typically regarded as posing a lower risk of toxicity to people and other creatures when compared to mercury, selenium, and chromium. It is likely that new information on the toxicity of molybdenum, which has been shown to inhibit spermatogenesis and arrest embryogenesis at levels as low as several parts per million [23,24] in organisms like catfish and mice, will likely prompt more research into the microbial molybdenum detoxification process in the near future.

The molybdenum reduction capability of the *Bacillus* sp. strain khayat has been described in the past. As a function of the starting molybdenum concentrations, the bacterial synthesis of molybdenum blue followed sigmoidal curves. [Case in point:] Previous works [12,25] have explored the kinetics of Mo-blue creation; nevertheless, in order to acquire the growth rate correctly for secondary modeling, they always resort to linearizing the production profile with time. This study aims to compare and contrast the Logistic [26,27], Gompertz [27,28], Richards [27,29], Schnute [27], Baranyi-Roberts [30], Von Bertalanffy [31,32], Buchanan three-phase [1], and most recently Huang model [3] (**Table 1**) models for predicting Mo-blue production in this bacterium. Nonlinear regression analysis of Mo-blue production has many benefits, so this study will

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

Model	p	Equation
Modified Logistic	3	$y = \frac{A}{1 + exp\left[\frac{4q_m}{A}(\lambda - t) + 2\right]}$
Modified Gompertz	3	$y = Aexp\left\{-exp\left[\frac{q_m \cdot e}{A}(\lambda - t) + 1\right]\right\}$
Modified Richards	4	$y = A\left\{1 + vexp(1+v)exp\left[\frac{q_m}{A}(1+v)\left(1+\frac{1}{v}\right)(\lambda - t)\right]\right\}^{\left(\frac{-1}{v}\right)}$
Modified Schnute	4	$y = \left(q_m \frac{(1-\beta)}{\alpha}\right)\left[\frac{1-\beta exp(\alpha\lambda + 1 - \beta - \alpha t)}{1-\beta}\right]^{\frac{1}{\beta}}$
Baranyi-Roberts	4	$y = N_0 + q_m t + \frac{1}{q_m} ln(e^{-q_m t} + e^{-h_0} - e^{-q_m t - h_0}) - ln\left[1 + \frac{e^{q_m t + \frac{1}{\mu} ln(e^{-q_m t} + e^{-h_0} - e^{-q_m t - h_0})}}{e^{(A - N_0)}}\right]$
Von Bertalanffy	3	$y = K\left[1 - \left[1 - \left(\frac{A}{K}\right)^3\right] exp^{-\left(\frac{\mu_m t}{3K}\right)^{\frac{1}{3}}}\right]$
Huang	4	$y = A + q_m - ln\left(e^A + (e^{q_m} - e^A)e^{-q_m B(t)}\right)$ $B(t) = t + \frac{1}{\alpha} ln \frac{1 + e^{-\alpha(t-\lambda)}}{1 + e^{\alpha\lambda}}$
Buchanan Three-phase linear model	3	Y = N_0, IF X < LAG Y= N_0+ K(X−λ), IF λ ≤ X ≥ Q_M Y = A, IF X ≥ Q_M

Note:
A= Metal reduction upper asymptote;
N_0= Metal reduction lower asymptote;
q_m= maximum specific metal reduction rate;
v= affects near which asymptote maximum metal reduction occurs.
λ=lag time
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.
For the Baranyi-Roberts model, the lag time (λ) (h^{-1}) or (d^{-1}) can be calculated as $h_0=\mu_m$
For modified Schnute, $A = \mu/\alpha$

MATERIALS AND METHODS

Isolation and maintenance of the Molybdate-reducing bacterium

Khayat et al. [33] were the ones that initially isolated the bacteria, determined its identity, and described it. Manuscript in preparation). The growth and maintenance were carried out on solid agar in low phosphate media with a pH of 6.5. The medium contained glucose at a concentration of 1 percent, $(NH_4)_2SO_4$ at a concentration of 0.3 percent, $MgSO_4.7H_2O$ at a concentration of 0.05 percent, NaCl at a concentration of 0.5 percent, yeast extract at a concentration of 0.0.5 percent, $Na_2MoO_4.2H_2O$ at a concentration of 0.242 percent, and Na [10]. Separate autoclaving was performed on the glucose.

Preparation of resting cells for molybdenum reduction characterization

Resting cells were utilized in a microplate or microtiter format to statically monitor Mo-blue production at various sodium molybdate concentrations, as was described before [34]. The only difference between the Low Phosphate Media and the High Phosphate Media was a fixed concentration of 100 mM for the HPM, and overnight cultures of 1 liters of High Phosphate Media were grown at room temperature on an orbital shaker rotating at 150 revolutions per minute. The cells were collected by

subjecting the sample to 10 minutes of centrifugation at 15,000 × g. Before being resuspended in 20 mL of low phosphate medium (LPM) without molybdenum, the pellet was washed multiple times in order to remove any phosphate that might have been present. The absorbance at 600 nm was adjusted to around 1.00. Higher doses were found to have a more pronounced inhibiting effect on molybdate reduction [10-12,14,35-39]. After that, an amount of 180 L was pipetted into each well of a microplate that had been cleaned. After that, 20 liters of stock solution with different amounts of sodium molybdate was poured into each well in order to initiate the creation of molybdenum blue. The tape was sealed using a sterile gas-permeable sealing tape (Corning® microplate), which allowed for gas exchange after it was applied. Incubations of microplates were carried out at ambient temperature (28 ºC). The absorbance at 750 nm was measured using a Microtiter Plate reader manufactured by BioRad (Richmond, California), and the readings were taken at specific intervals (Model No. 680). Mo-blue production was quantified by measuring the specific extinction coefficient of the medium at 750 nm, which was the longest wavelength for which a filter was available for the microplate unit [40]. This was done since 750 nm was the longest wavelength for which a filter was available.

Determination of Kinetic Parameters for Molybdenum Blue production

Fitting of the data

Nonlinear regression was used to fit the growth data to the nonlinear equations, and the Marquardt algorithm, which minimizes sums of squares of residuals, was utilized in the process with the assistance of the CurveExpert Professional program. Nonlinear regression was used to fit the data, and nonlinear equations were used (Version 1.6). This lookup method's goal is to reduce, as much as possible, the square of the difference that exists between the values that were anticipated and those that were actually observed. Programming the software in question allows for the calculation of initial values of parameters to be carried out either automatically or manually. In order to estimate m, we searched for the point on the curve that had the steepest rise between four reference sites. Discovering the point at which this line cuts through the x-axis gave us the ability to construct a rough estimate for. The asymptote, denoted by "A," was computed by making use of the most recent data point. Because of the structure of the differential equation that makes up Huang's model, the solution to this equation must be found through the use of numerical methods. Through the application of the Runge-Kutta method, the differential equation was given a numerical solution. In order to find a solution to this issue, we consulted the ode45 solver in MATLAB (Version 7.10.0499, The MathWorks, Inc., Natick, MA).

Statistical analysis

The ability of models with different numbers of parameters to account for the same set of experimental data was evaluated using a variety of methods, such as the corrected Akaike Information Criterion (AICc), the Root-mean-square error (RMSE), the bias factor (BF), the accuracy factor (AF), and the adjusted coefficient of determination (adjR^2). We used equation (1) to calculate the root-mean-square error, where Ob_i stands for the experimental data, Pd_i stands for the model-predicted values, n stands for the number of experimental data, and p stands for the number of parameters in the assessed model. For the model with fewer parameters, it is anticipated that the RMSE values will be lower [41].

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

The R^2 statistic, also known as the coefficient of determination, is used in linear regression to evaluate how well a model fits the data. However, using the R2 method in nonlinear regression does not readily produce comparable analyses because the number of parameters varies from model to model. This is one of the reasons why using the R^2 method is not recommended. Calculating the quality of nonlinear models with equations 2 and 3 and the updated R^2 using the formula where is the total variance of the y-variable and RMS is the residual mean square is one way to tackle this problem. Another way to address this problem is to calculate the residual mean square.

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

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

Information Criterion of Akaike, often known as The Akaike Information Criterion (AIC) is what takes care of the trade-off between the complexity of the model and its goodness of fit. The AIC gives a solution to the problem of model selection [42], which it does by determining the relative quality of a statistical model for each collection of experimental data that is provided. In point of fact, it is founded on a body of knowledge known as "information theory." When a certain model is used to represent the process that generates the information or data in question, this method provides a rough estimate of the amount of data that is lost as a result of this choice. For any particular set of predicted results, the model that has the lowest value for the Akaike Information Criterion (AIC) will be the one that is most universally accepted by the scientific community. This is typically a negative number; for instance, a value of -10 is preferred above a value of -1 as an illustration of this preference. To put it another way, the AIC value will increase if there are more parameters because the result will be less desirable when there are more parameters. As a consequence of this, AIC not only promotes the use of a model that is less complex (underfitting) in order to accommodate experimental data, but it also rewards quality of fit. When there are fewer values to consider or more parameters to take into account, the Akaike information criterion (AIC) with correction (AICc) is utilized in place of the AIC [43]. We are able to calculate the AICc for each dataset and model by utilizing the formula that is provided below (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 n refers to the total number of points on the curve and p refers to the total number of parameters in the model. The strategy takes into consideration both the change in goodness-of-fit as well as the variation in model parameters. The model that has the smallest value for the AICc statistic is the one that is most likely to be accurate for each dataset [43]. The Accuracy Factor (AF) and the Bias Factor (BF) (**Eqns. 5** and **6**) were also employed to measure the goodness-of-fit of the models. Both of these factors were first presented by Ross [44]. There is a perfect correlation between the values that were observed and those that were anticipated when the Bias Factor equals 1. Studies of microbial growth curves or Mo-blue production in which the bias

factor is less than 1 indicate a potentially unsafe model, whereas studies in which the bias factor is more than 1 indicate a safe model. The typical value of the Accuracy Factor is 1, and higher values suggest a less precise or accurate prediction than the average value.

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

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

RESULTS AND DISCUSSION

There was a discernible increase in the amount of blue coloration after 50 h of static incubation. The Mo-blue production from this bacterium had the shape of a sigmoidal curve, with a lag period of around 15 h and attaining maximum Mo-blue production (**Fig. 1**). The Mo-blue output vs. time profile was installed in a total of eight different automobiles. Produces an appearance that is agreeable to the eye (**Fig. 2**). The most recent revision of the logistics model yielded the best results, with the best adjusted R^2 value as well as the lowest RMSE and AICc values. Both the AF and BF of the model had values that were extremely close to 1.0, indicating that they were highly good. The Baranyi-Roberts model obtained the lowest possible results in the vast majority of statistical evaluations (**Table 2**). The updated logistics model coefficients for a variety of molybdenum concentrations are presented in **Table 3**, where they can be found.

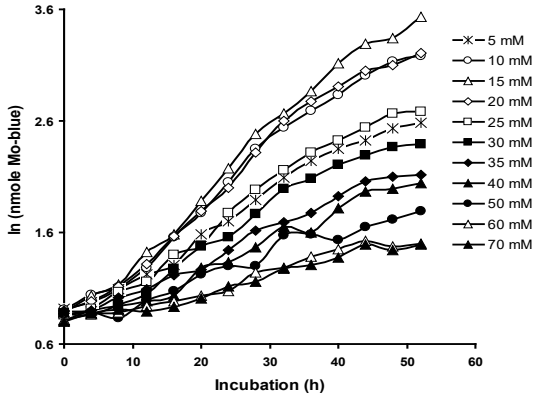

Fig. 1. *Bacillus* sp. strain khayat's Mo-blue production curves at different sodium molybdate concentrations over time. Mean SD from three independent measurements are shown as error bars.

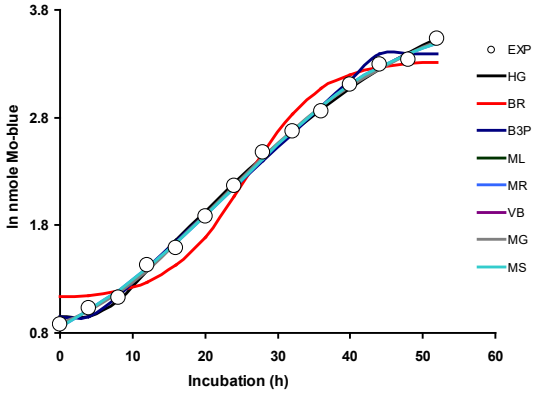

Fig. 2. After fitting to several different models, this is the Mo-blue production curve for *Bacillus* sp. strain khayat growing in 25 mM sodium molybdate. The following models were used in the study: Huang (HG), Baranyi-Roberts (BR), Buchanan (B3P), modified Logistics (ML), modified Richards (MR), von Bertalanffy (VB), modified Gompertz (MG), and modified Schnute (MS).

Table 2. Statistical analysis of the various fitted models.

Model	*p*	RMSE	adR^2	AF	BF	AICc
Huang	4	0.0568	0.996	1.0270	0.9991	-59.50
Baranyi-Roberts	4	0.1764	0.958	1.0774	1.0081	-27.79
modified Gompertz	3	0.1666	0.964	1.0161	1.0003	-35.12
Buchanan-3-phase	3	0.0729	0.993	1.0291	1.0013	-58.27
modified Richards	4	0.0438	0.998	1.0178	0.9994	-66.79
modified Schnute	4	0.1778	0.959	1.0178	0.9994	-27.58
modified Logistics	3	0.0488	0.997	1.0228	0.9974	-69.50
von Bertalanffy	3	0.0750	0.993	1.0363	0.9968	-57.46

Note:
p no of parameters
adR² Adjusted Coefficient of determination
RMSE Root Mean Square Error
BF Bias factor
AF Accuracy factor

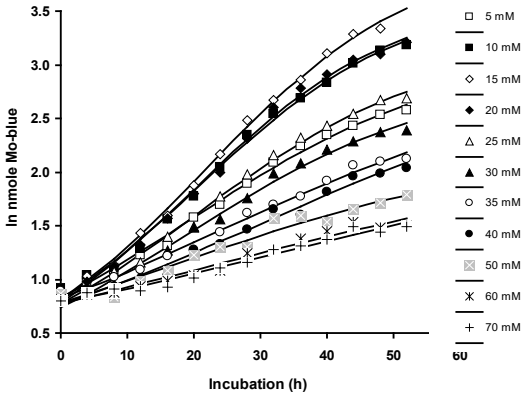

Fig. 3. The Mo-blue production curves of *Bacillus* sp. strain khayat were fitted using the modified logistics model. These curves were based on varied concentrations of sodium molybdate.

Table 3. Mo-blue production coefficients at various molybdenum concentrations as modelled using the modified logistics 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)	3.14	3.664	4.047	3.65	3.203	2.863	2.863	3.053	2.234	2.185	2.618
μ_m (h^{-1})	0.04	0.057	0.064	0.06	0.045	0.039	0.039	0.027	0.022	0.016	0.015
Lag (h)	19.21	11.78	9.82	10.80	14.98	17.07	17.07	25.25	35.69	47.72	49.43

The logistics model is amongst the first to be developed for modelling microbial growth. The growth rate according to the model is given by a differential equation as follows:

$$\frac{dA}{dt} = \mu_m A\left(1 - \frac{A}{A_{\max}}\right)$$

where the maximal specific growth rate, denoted by the letter m, can be calculated by taking the product of the initial population density, at time t (optical density), and the number of bacterial cells, expressed as CFU per milliliter. At the stationary phase, the value marked by the letter Amax represents the maximum population density of bacteria, also known as the optical density or CFU/mL. This limit is also referred to as the carrying capacity of the planet, which is a common statement. When the optical density of a population or the number of viable bacteria in a given volume of medium is very high, the term 1-A/A_{max} in the logistics model inhibits growth. This happens when the optical density of a population is very high. When A is very small, as it is when the lag phase is occurring, the term is practically turned to one, and as a result, it has very little impact on the growth rate. At high population density (optical density) or bacterial cell number (CFU/mL), the value of A approaches

A_{max}, changing the term to virtually zero, which leads to a nearly zero growth rate at the stationary phase. This is the case because A_{max} is the maximum value of A. The sigmoid curve is the form that was produced as a result. Gibson et al. [45] made a number of modifications to the logistic model in order to make it compatible with the data on the development of bacteria.

$$\log A = a + c / [1 + \exp(-b(t-m))]$$

This is an example of an exponential function, and the a, c, b, and m parameters are as follows: The fact that the logistics model has been utilized to successfully imitate the growth of bacteria as well as the manufacturing of products derived from other organisms [46-48,48-50] is evidence that the logistics model is adaptable. The fitting technique resulted in the calculation of three parameters: specific reduction rate (h^{-1}), Lag period (h), and maximum Mo-blue production (nmole Mo-blue). Utilizing models such as the two-parameter Monod model or other, more advanced "secondary models" such as Haldane, Aiba, Yano, and others would allow secondary modeling of Mo-blue synthesis to take use of these physiologically significant coefficients. The purpose of the mechanistic models that are used in fundamental research is to gain a deeper understanding of the underlying physical, chemical, and biological processes that are responsible for the observed growth profile. If we make the assumption that all other parameters are the same, mechanistic models are preferable to other types of models because they offer information about the underlying mechanisms that are responsible for observable patterns. When their applicability is projected beyond the current circumstances, the likelihood of their being correct increases [51].

CONCLUSION

It was determined through a series of statistical tests or error function analysis such as root-mean-square error, adjusted coefficient of determination, bias factor, accuracy factor, and corrected Akaike information criterion, that the logistics model accurately modelled Mo-blue production curve from the bacterium *Bacillus* sp. strain khayat. A review of the relevant literature indicates that the parameters obtained from the fitting exercise will be of great use in the subsequent development of the secondary model, which will be innovative not only for the conversion of molybdenum to Mo-blue but also for the detoxification of heavy metals in general. As part of the secondary modeling of Mo-blue production from this bacterium, the modeling of the inhibitory influence of the substrate molybdenum on the maximum Mo-blue production rate values derived from this work is currently under progress. In addition to modeling other parameters, such as the influence of environmental variables, modeling is being done on Mo-blue production rates (pH and temperature).

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