

Statistical Evaluation of the Mathematical Modelling on the Molybdenum Reduction Kinetics of a Molybdenum-reducing Bacterium

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ABSTRACT

Molybdenum is a global pollutant that has been increasingly found to be toxic to spermatogenesis in several animal model studies. Thus its removal and the studies of its reduction kinetics for removal are very important. In a previous study (1), three kinetic models; i.e. Haldane, Luong and Monod were evaluated to study the reduction kinetics of the molybdenum-reducing bacterium *Bacillus* sp. strain A.rzi. We revisited the model used in this bacterium and extend the study by incorporating more reduction kinetics models such as Hans-Levenspiel, Yano, Aiba, Webb, Hinshelwood and Teissier. We then evaluated the accuracy of the fitted model using statistical analysis. The best model was Han–Levenspiel instead of Luong as proposed previously with the majority of the statistical evaluation such as the lowest values for RMSE, the highest adjusted R^2 values and with Bias Factor and Accuracy Factor nearest to unity (1.0) indicated that the model was the best. The calculated values for the Han-Levenspiel constants q_{max} (the maximum specific substrate reduction rate (h^{-1}), S_m (the critical inhibitor concentration (mM) above which reactions stops), K_s (concentration of substrate giving half maximal reduction rate (mM) and the empirical constants m and n (both dimensionless) were 3.832, 106.16, 48.19, -1.271 and 1.001, respectively. The true q_{max} value where the gradient for the slope is zero was 1.107 h^{-1} at 38 mM molybdate as a substrate. The results indicate that the exhaustive use of mathematical models provides new understanding of the way molybdenum inhibits production into molybdenum blue in bacterium. This can provide new knowledge on ways to predict progress of molybdenum reduction in the laboratory and during bioremediation works.

INTRODUCTION

Molybdenum level as low as several parts per million is very toxic to cows causing scouring and deaths when exposed to this heavy metal [1,2]. More recently, molybdenum at the same trace level has been discovered to be very toxic to spermatogenesis in catfish and mice [3–6]. It is generally accepted that in many cases bioremediation is green alternative in removing toxic metals including molybdenum from soils and water bodies [7]. Although many molybdenum reducing bacteria have been isolated [8–19] their reduction kinetics is one

of the area that is least studied. To date, only one study has been carried out in *Bacillus* sp. strain A.rzi and the best model was Luong [11] indicating that at high concentration of molybdenum, reduction rates are inhibited. The use of extensive models available could reveal that in certain circumstances other reduction models could be more appropriate.

Hence, the objective of this work is to comprehensively evaluate Mo-reduction kinetics in this bacterium and to evaluate the best model with statistical analysis support. The results

could be used for further secondary modelling studies including the effect of pH and temperature on reduction kinetics. This should give new data and results that could spurn and reveal new information and improvement in the works already done.

Table 1. Various mathematical models developed for reduction kinetics involving substrate inhibition.

Author	Reduction Rate	Author
Monod	$q_{\max} \frac{S}{K_s + S}$	[20]
Haldane	$q_{\max} \frac{S}{S + K_s + \frac{S^2}{K_i}}$	[21]
Teissier	$q_{\max} \left(1 - \exp\left(-\frac{S}{K_i}\right) - \exp\left(-\frac{S}{K_s}\right) \right)$	[22]
Aiba	$q_{\max} \frac{S}{K_s + S} \exp(-KP)$	[23]
Yano and Koga	$\frac{q_{\max} S}{S + K_s + \left(\frac{S^2}{K_i} \right) \left(1 + \frac{S}{K} \right)}$	[24]
Han and Levenspiel	$q = \frac{q_{\max} S \left[1 - \left(\frac{S}{S_m} \right) \right]^n}{K_s + S - \left[1 - \left(\frac{S}{S_m} \right) \right]^n}$	[25]
Luong	$q_{\max} \frac{S}{S + K_s} \left[1 - \left(\frac{S}{S_m} \right) \right]^n$	[26]
	$q_{\max} S \left(1 + \frac{S}{K} \right)$	
Webb	$S + K_s + \frac{S^2}{K_i}$	[22]
Hinshelwood	$q_{\max} \frac{S}{K_s + S} (1 - KP)$	[27]

Note:

q_{\max} maximal reduction rate (h^{-1})
 K_s half saturation constant for maximal reduction (mM)
 S_m maximal concentration of substrate tolerated and (mM)
 m, n, K curve parameters
 S substrate concentration (mM)
 P product concentration (mM)

MATERIALS AND METHODS

Chemicals

All chemicals used were of analytical grade. Buffers were prepared at the appropriate temperature according to their final use by mixing the appropriate basic and dibasic salts as outlined by Dawson et al. [28].

Determination of Kinetic Parameters for Molybdate Reduction to Molybdenum Blue

Determination of intrinsic growth kinetic parameters for this bacterium was not possible due to the property of the molybdenum blue that form a precipitate together with the bacterial mass [9,17,18]. Hence, only the reduction kinetics was studied. In this work data for the molybdenum reduction kinetics represented as Mo-blue production rate (\square mole Mo-blue/h) was sourced from Othman et al. [11].

Fitting of the data

The nonlinear equations were fitted to growth data by nonlinear regression with a Marquardt algorithm that minimizes sums of square of residuals using CurveExpert Professional software (Version 1.6). This is a search method to minimize the sum of the squares of the differences between the predicted and measured values.

Statistical analysis

To decide whether there is a statistically substantial difference between models with different number of parameters, in terms of the quality of fit to the same experimental 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) [29,30].

The RMSE was calculated according to Eq. (2), 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.

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

Adjusted R^2 is calculated according to the following formula where RMS is Residual Mean Square and s_y^2 is the total variance of the y-variable.

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

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

The AICc is calculated for each data set for each model according to the following equation;

$$AICc = 2p + n \ln \left(\frac{RSS}{n} \right) + 2(p + 1) + \frac{2(p + 1)(p + 2)}{n - p - 2} \quad (4)$$

Where n is the number of data points and p is the number of parameters of the model. The method takes into account the change in goodness-of-fit and the difference in number of parameters between two models. For each data set, the model with the smallest AICc value is highly likely correct. Accuracy Factor (AF) and Bias Factor (BF) to test for the goodness-of-fit of the models as suggested by Ross [31] were also used. The equation is as follows;

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

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

RESULTS AND DISCUSSION

The results of the curve fitting are shown in **Figs. 1 to 7**. Models such as Monod and Hinshelwood failed to fit the experimental data and were omitted. All of the other models tested gave reasonably good fitting based on visual observation.

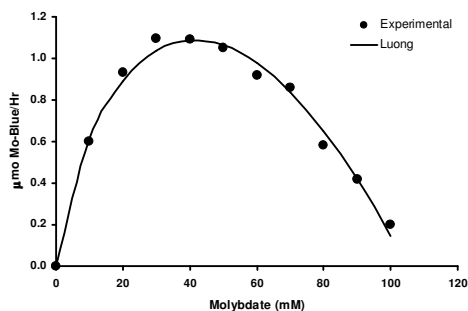


Fig. 1. Fitting experimental data with the Luong model.

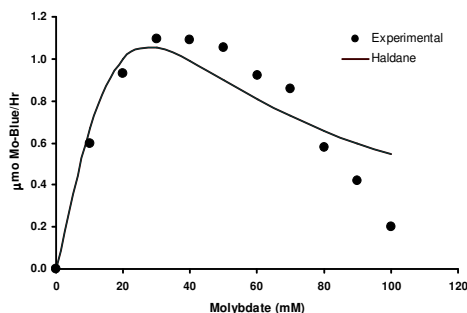


Fig. 5. Fitting experimental data with the Haldane model.

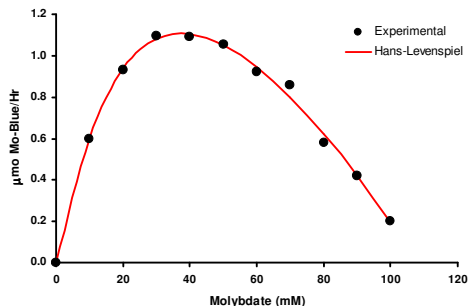


Fig. 2. Fitting experimental data with the Han-Levenspiel model.

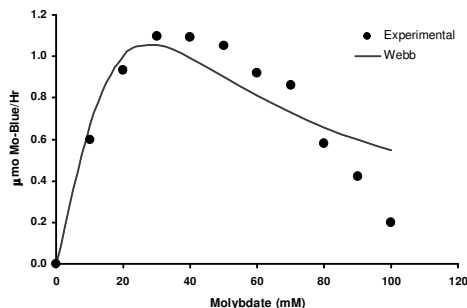


Fig. 6. Fitting experimental data with the Webb model.

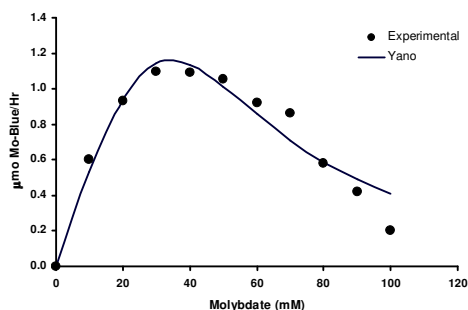


Fig. 3. Fitting experimental data with the Yano model.

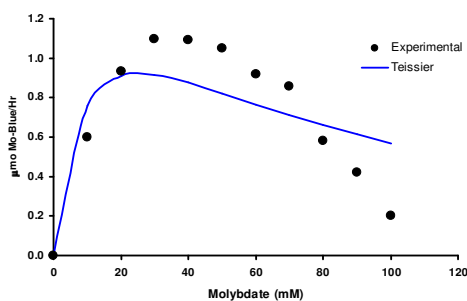


Fig. 7. Fitting experimental data with the Teissier model.

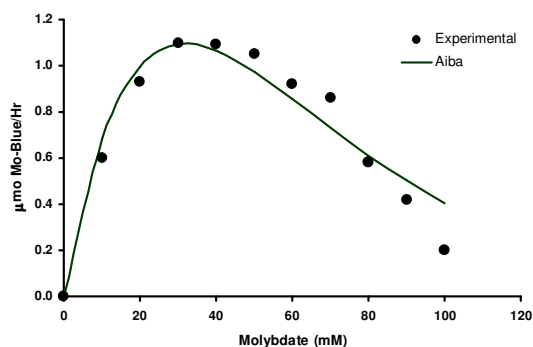


Fig. 4. Fitting experimental data with the Aiba model.

Statistical analysis (Table 2) shows that the best model was Han–Levenspiel instead of Luong as proposed previously with the majority of the statistical evaluation such as the lowest values for RMSE, the highest adjusted R^2 values and with Bias Factor and Accuracy Factor nearest to unity (1.0) indicated that the model was the best.

Table 2. Statistical analysis of kinetic models.

Model	p	SSE	MSE	RMSE	R^2	ad R^2	AICc	BF	AF
Luong	4	0.0169	0.0024	0.0491	0.988	0.980	-41.26	0.99	1.03
Han-Levenspiel	5	0.0065	0.0011	0.0330	0.995	0.991	-38.69	1.00	1.01
Yano	4	0.0856	0.0122	0.1106	0.930	0.884	-23.41	1.03	1.06
Aiba	4	0.0894	0.0128	0.1130	0.919	0.864	-22.94	1.04	1.07
Haldane	3	0.2335	0.0292	0.1708	0.733	0.619	-21.71	1.05	1.11
Webb	4	0.2336	0.0334	0.1827	0.733	0.555	-12.37	1.05	1.11
Teissier	3	0.3821	0.0478	0.2186	0.422	0.174	-16.29	1.04	1.13

Note:

P No of parameters

SSE Sums of Squared Errors

RMSE Root Mean Squared Error

 R^2 Coefficient of Determinationad R^2 Adjusted Coefficient of Determination

AICc Corrected Akaike Information Criterion

BF Bias Factor

AF Accuracy Factor

The calculated values for the Han-Levenspiel constants q_{max} (the maximum specific substrate reduction rate (h^{-1}), S_m (the critical inhibitor concentration (mM) above which reactions stops), K_s (concentration of substrate giving half maximal reduction rate (mM) and the empirical constants m and n (both dimensionless) were 3.832, 106.16, 48.19, -1.271 and 1.001, respectively. It needs to be cautioned that the q_{max} value obtained based on curve fitting interpolation is not the true value as the true q_{max} should be where the gradient for the slope is zero and in this case (Han-Levenspiel) the value was approximately 1.107 h^{-1} at 38 mM molybdenum (Fig. 2). The equation for the Han-Levenspiel using the values obtained from the fitting (Eqn. 7 and 8) is as follows;

$$q = 3.38 \frac{S}{S + 115.8} \left[1 - \left(\frac{S}{57.83} \right)^{1.405} \right] \quad (7)$$

$$q = \frac{1.107 S \left[1 - \left(\frac{S}{106.16} \right) \right]^{1.001}}{48.19 + S - \left[1 - \left(\frac{S}{106.16} \right) \right]^{-1.271}} \quad (8)$$

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.

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 [29]. AIC handles the trade-off relating to the goodness of fit of the model as well as the complexity of the model. It is actually established on information theory. The method provides a relative approximation of the information lost for each time a given model is utilized to represent the process that creates the information or data. For an output of a set of predicted model, the most preferred model would be the model showing 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 equation incorporates number of parameters penalty, the more the parameters, the less preferred the output or the higher the AIC value. Hence, AIC not merely rewards goodness of fit, but in addition does not encourage using more complicated model (overfitting) for fitting experimental data. Since the data in this work is small compared to the number of parameter used a corrected version of AIC, the Akaike information criterion (AIC) with correction or AICc is used instead.

The Bias Factor equal to 1 indicate 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. Most of the studies concerning substrate inhibition on microbial growth are carried out using toxic substrates such as aromatic and halogenated hydrocarbons and elements such as mercury, chromium and molybdenum [11,32,33] and hence it can be deduced that at high concentration growth rate will be severely affected and the normal use of the Monod model will fail. There were other models for describing substrate inhibition kinetics developed during this period such as the discontinuous models

of Wayman and Tseng [34]. The reason for the development of the discontinuous model is the previous models developed such as Haldane, Andrews and Noack, and Webb can describe inhibitory effect on microbial growth but could not explain or adequately model for certain situations where the growth rate completely ceased or becoming zero at very high substrate concentration. However, the discontinuous fitting profile of the Wayman and Tseng model is a major drawback [35]. A continuous version of the above models developed by Luong have found popular support due to its close agreement to experimental data in a number of cases [11,36,37] including the result obtained on this bacterium from the previous paper. A central attraction of the Luong model is its ability to successfully predicting the value of S_m , the maximum substrate concentration above which growth is completely inhibited. The results from this work showed that the Luong model is second best. The Han-Levenspiel model is a generalized form of Monod kinetics. It is proposed to take into account for all possible cell, product and substrate inhibition. The heart of the model is an assumption that there exists a point or a critical inhibitor concentration above which cells cannot grow similar to the Luong assumption, and that the Monod equation constants are functions of this critical inhibitor concentration [25].

Most studies on the reduction kinetics of heavy metals such as mercury [38], arsenate [39] and chromate [40] reported a Haldane-type inhibition by the substrate metal ions. However, molybdenum reduction to Mo-blue showed a clear strong inhibition of Mo-blue production rate at high concentration of molybdenum with a distinct critical concentration of molybdenum that completely inhibited Mo-blue production. Unlike the more commonly reported Haldane model [41], both the Luong and the Han-Levenspiel models allow for the determination of the critical concentration of substrate that could completely inhibited production of product as evident from this work. This is the first time the Han-Levenspiel model being applied to model Mo-blue production in bacterium

CONCLUSION

Both growth and reduction kinetics of bacteria can be modelled using various models available in the literature. Literature survey has shown that for the same compound, various models have been found optimum in different systems and hence a comprehensive modelling exercise was carried out on available published works to demonstrate this observation. In this work, we demonstrated based on statistical analysis that the Han-Levenspiel model is better than Luong in fitting the reduction kinetics data *Bacillus* sp. strain A.rzi.

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