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Mathematical models

Inhibition Kinetics Study of Molybdenum Reduction by *Pantoea* sp. strain HMY-P4

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ABSTRACT

Physical, chemical or biological data mathematical simulation may assist the investigator to describe a phenomenon found based on physical, chemical or biological mechanisms. The model may also be used to forecast or predict future behavior, simulate a hypothetical event or feedback, and better tests can be planned. Molybdenum reduction to the insoluble molybdenum blue by bacteria is an emerging tool for bioremediation of molybdenum pollutant worldwide. In this study, the molybdenum reduction rate by the bacterium *Pantoea* sp. strain HMY-P4 was studied for its inhibitory kinetics characteristics using sodium molybdate as a substrate. Modeling kinetics showed that molybdenum reduction could be explained by several models such as Monod, Haldane, Hans-Levenspiel, Luong, Teissier, Aiba and Yano with Aiba as the best model as judged using error function analysis such as bias and accuracy factors (BF and AF), root mean square error (RMSE), adjusted coefficient of determination (adjR^2), and corrected Akaike Information Criterion (AICc). The calculated values for the Aiba constants q_{max} (the maximum specific substrate degradation rate (h^{-1}), K_s (concentration of substrate at the half maximal degradation rate (mg/L) or saturation constant and K_i (inhibition constant (mg/L)) were 0.060 (95% CI, 0.024 to 0.096), 0.302 (95% CI, 0.381 to 0.222) and 0.953 (95% CI, 0.568 to 1.338), respectively. These model parameters indicate a good tolerance of the bacterium against high concentration of toxic molybdenum making the bacterium useful for bioremediation works.

INTRODUCTION

Bioremediation is one way to eliminate harmful heavy metals such as molybdenum from the atmosphere using a microorganism. Molybdenum is an emerging pollutants of global concern [1]. Microorganisms are theoretically used quicker, easier and safer for the removal of molybdenum than traditional and physical approaches especially in cases of soil pollution where these other methods will be less effective or highly costly [2]. Molybdenum remediation on areas polluted by molybdenum was effective in mitigating toxic effects of molybdenum using a bacteria consortium on bovine pasture lands in Tyrol, Austria [3]. Molybdenum is one of the essential trace elements that is necessary for more than 50 enzymes and acts as a micronutrient [4]. It helps to facilitate cellular activity in animal and plant physiology, with the catalytic production of a combination of redox and hydroxylation exchange. Earlier experiments have demonstrated that in many animal model's molybdenum disrupts endocrine function. Several molybdenum reduction bacteria have been isolated before recently with the potentially to co-degrade other organic contaminants [5–10]. A more detailed understanding of the mechanism of reduction and kinetics of the Mo-reducing enzyme through different processes of optimization would also help to overcome issues in

molybdate reduction to Mo-blue. In *Bacillus* sp A.rzi and *Serratia* sp. MIE2, mathematical modelling performed on hexavalent molybdenum reduction to Mo-blue suggest that the best models were the Luong and Teissier models, respectively [11,12]. The Luong and Teissier models, despite the widely recorded Haldane model allow for the determination of crucial substrate concentration, which can fully inhibit the rate of bacterial processes [13,14].

Literature search shows that a Haldane-type inhibition is reported in several metal reduction kinetics studies such as mercury (Głuszcz *et al.,* 2011), arsenate (Soda *et al.,* 2006) and chromate (Sukumar, 2010), while in the bacterial reduction of uranium, a Monod model is reported (Truex *et al.,* 1997). Thus, this secondary modeling operation can also be used to assess whether the substrate is not inhibitory to the reduction rates (Monod) or inhibitory (Haldane, Teissier, Aiba, Yano and Luong).

MATERIALS AND METHOD

Bacterium and culture media preparation

The bacterium used in this study was previously isolated and identified as *Pantoea* sp. strain HMY-P4 [15]. All media preparations (solid and broth) were made according to the recipe of Shukor *et al.* [16] except otherwise stated.

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

Author	Degradation Rate	Author
Monod	$q_{\max}\frac{S}{K_s+S}$	[18]
Haldane	$q_{\max}\frac{S}{S+K_s+\frac{S^2}{K_i}}$	[19]
Teissier	$q_{\max}\left(1-\exp\left(-\frac{S}{K_i}\right)-\exp\left(\frac{S}{K_s}\right)\right)$	[20]
Aiba-Edward	$q_{\max}\frac{S}{K_s+S}\exp\left(\frac{-S}{K_i}\right)$	[20,21]
Yano and Koga	$\frac{q_{\max}S}{S+K_s+\left(\frac{S^2}{K_1}\right)\left(1+\frac{S}{K}\right)}$	[22]
Edward (Webb)	$q_{\max}\frac{S\left(1+\frac{S}{K}\right)}{S+K_s+\left(\frac{S^2}{Ki}\right)}$	[20]
Luong	$q_{\max}\frac{S}{S+K_s}\left[1-\left(\frac{S}{S_m}\right)^n\right]$	[14]

Note:
q_{max} maximal degradation rate (h^{-1})
K_s half saturation constant for maximal reduction (mg/L)
S_m maximal concentration of substrate tolerated and (mg/L)
m, n, K curve parameters
S substrate concentration (mg/L)

Low phosphate-molybdate medium (LPM) and agar

The medium was prepared $(NH4)_2SO_4$, 3 g, $MgSO_4.7H_2O$, 0.5 g, NaCl, 5 g, $Na_2MoO_4.2H_2O$, 2.42g, Na_2HPO_4, 0.71g, yeast extract, 0.5 g and glucose, 10 g into a liter of deionized water. The pH was adjusted to pH 7.5 prior to autoclaving at 121 °C, 115 kPa for 15 min. Glucose must be autoclaved separately and added once the medium cooled sufficiently. For preparation of plate agar, 8 g of agar was added to the medium prior to autoclaving. Glucose was separately autoclaved and added to the medium afterwards. Mo-blue produced from the fermentation was quantified at 865 nm using the extinction coefficient value of 16.7 mM^{-1} cm^{-1} [17].

Modeling kinetic experiment

Molybdenum reduction kinetic was carried out according a previous method [12]. Batch experiment (100 mL) was carried out in 250 mL conical flask but the initial molybdate concentration was varied from 0 to 100 mM. The Mo-blue produced was determined by measuring at 865nm of a 3 mL aliquot every 2 h until 24 h. In this study, six kinetic models are available in literature were used to represent the kinetics of molybdenum reduction which are listed in **Table 1**. All the seven kinetic models are fitted to the experimental data. The model parameters are evaluated by using the curve fitting software CurveExpert (v 1.6).

Error function analysis

A variety of methods for example corrected AICc (Akaike Knowledge Criterion), Root-Mean-Square Error (RMSE), bias factor (BF), accuracy factor (AF) and modified or adjusted coefficient of determination (R^2) were used to define a substantial difference between experimental data and modelled data. These included the use of a different number of parameters with regard to the consistency of fit to experimental results.

The RMSE was calculated based on Eqn. 1. The lower number of parameters of the model is supposed to yield a smaller RMSE value [23].

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

Where
Ob_i is the experimental data,
Pd_i is the values predicted by the model,
n is the number of experimental data and
p is the number of parameters of the assessed model

The coefficient of determination or R^2 is used to determine the fit consistency of the model in linear regression. Although the disparity in the number of parameters between one model and another varies in nonlinear regression, the use of the R^2 approach does not however, offer a comparable analysis. Therefore, the adjusted R^2 is used to calculate the quality of nonlinear models using **Eqns. 2** and **3** according to the formula

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

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

Where

s_y^2 is the total variance of the y-variable,

RMS is Residual Mean Square

In addition, the Akaike Information Criterion (AIC) is capable to calculate the relative quality of a given statistical model for any given set of experimental data thus offers a solution to model selection [24]. In reality, AIC can also resolve the trade-off in terms of experimental and sophistication of the model. This has been proved with respect to information theory. For example, the approach proposes a comparative estimate of the information lost each time the model used to describe the process when it produces the data. The performance of a series of projected models will be chosen as the most accepted one. This model would then be the model that indicates the minimal value for AIC. In general, a negative value is shown for this; an AICc value of-10 is the better model rather than that of -1. The calculation contained a set of penalty parameters. The corrected variant of AIC, the Akaike Knowledge Criteria (AIC) with correction or AICc is preferred to be used in data with a lower number of values or a higher number of parameters used.[25]. The AICc is calculated for each data set for each model according to the following equation (**Eqn. 4**);

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

(**Eqn 4**)

Where
n is the number of data points,
p is the number of parameters.

Both Accuracy Factor (AF) and Bias Factor (BF) that were used to test the goodness-of-fit of the models were calculated according to **Eqns. 5** and **6** as suggested by Ross [26]. A Bias Factor that is equal to 1 show an ideal match between observed and predicted values. Within microbial growth curves or Mo-blue production studies, a bias factor with the value < 1 indicates a fail-dangerous model whereby a bias factor with the value > 1 indicates a model that is fail-safe. In a case where the value of the Accuracy Factor is frequently ≥ 1 along with higher AF values, the prediction that is said to be less precise or accurate.

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

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

Where
Ob_i is the experimental data,
Pd_i is the values predicted by the model,
n is the number of experimental data and

RESULTS AND DISCUSSION

The effect of different concentrations of molybdate to molybdenum reduction after 24 h of incubation shows that molybdenum blue production increases until 20 mM but starting to decrease at higher concentrations.

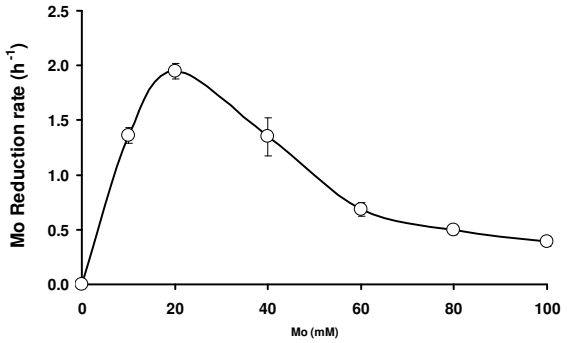

Fig. 1. The effect of sodium molybdate as a substrate to molybdenum reduction by the bacterium. Error bars represent mean ± standard deviation (n=3).

In this work, molybdenum reduction kinetics is represented as Mo-blue production rate where q_{max}, K_s, K_i, S, S_m, are specific Mo-blue production rate (hr^{-1}), maximum Mo-blue production rate (hr^{-1}), half-saturation constant (mM), inhibition constant(mM), substrate concentration (mM), critical substrate concentration above which production of Mo-blue completely stops (mM), and K and n are Yano constants (mM) and the exponent representing the impact of the substrate to q_{max}, respectively.

Data from the experimental values in batch studies was fitted to seven kinetic models using the software CurveExpert (v1.6) to find the constants (**Figs. 2 to 9**). Of the models, only the Luong, Yano, Aiba and Han-Levenspiel appeared to be visually acceptable in their fittings of experimental data whilst other models appear to be inadequate. The best model as judged by statistical analysis is the Aiba model based on lowest values for RMSE, AICc, adjR^2, BF and AF values closest to 1.0 and the second best is the Yano model (**Table** 2).

Table 2. Statistical analysis of the various models utilized to model Mo-blue production rate from *Pantoea* sp. strain HMY-P4.

Model	p	RMSE	adjR^2	AICc	BF	AF
Luong	4	1.370	0.912	47	0.066	15.127
Yano	4	0.088	0.975	4	0.970	1.053
Tessier-Edward	3	0.385	-0.972	8	1.022	1.195
Aiba	3	0.068	0.984	-20	0.976	1.064
Haldane	3	0.468	-0.486	11	1.110	1.370
Monod	2	0.552	-2.798	4	0.976	1.505
Han and Levenspiel	5	0.087	0.968	59	0.976	1.505

Note:
p no of parameters
adR² Adjusted Coefficient of determination
BF Bias factor
AF Accuracy factor
AICc Adjusted Akaike Information Criterion

The calculated value for the Aiba's constants, which are q_{max}, K_s, and K_i that are maximal reduction rate, half saturation constant for maximal reduction, half saturation constant for inhibition of reduction were 8.26±3.28 µmole Mo-blue hr^{-1}, 33.16±4.12 mM and 34.14±17.02 mM, respectively. The true maximal reduction rate, which occurred when the slope of the curve is zero (Arutchelvan *et al.,* 2006) occurs at 20 mM molybdate concentration and a corresponding value of 1.66 µmole Mo-blue hr^{-1}. The high variation in the values observed indicate the data modelled were not of high quality.

The Aiba model has been used in many biological processes with good success. Examples include amonium and nitrate oxidation rate in a suspended biomass system (SBS) in the nitratation processes in an immobilised biomass system [27] and the bioconversion of wastewater by the photosynthetic bacteria [28]. The Aiba model was also used in modelling the hydrogen production by cyanobacteria [29] and the rate of quinoline biodegradation and mineralization by an internal loop photo-biodegradation reactor [30]. It is also the best model in modelling manganese oxidation rate by *Streptomyces violarus* strain SBP1 [31] and the inhibitory effect of ethanol on ethanol fermentation by *Kluyveromyces* sp. IIPE453 [32]. Other examples include in the glyphosate degradation rate by *Bacillus subtilis* [33], the degradation rate of tributyl tin by *Klebsiella* sp. [34], nonyphenol biodegradation [35], cresol biodegradation [36] and crude glycerol fermentation to dihydroxyacetone by immobilized *G. oxydans* cells [37].

In the reduction of molybdenum to Mo-blue by *Bacillus* sp. Strain A.Rzi the Luong was the best model followed by Haldane and Monod. Luong was also the best model to fit Mo-blue production rate curve for the bacterium *Bacillus* sp. strain Lbna with q_{max}, K_s, S_m, and n values of 27.3 hr^{-1}, 115.8 mM, 57.83 mM and 1.405, respectively (Halmi *et al.,* 2014). In the bacterium *Serratia marcescens* Strain MIE2, rate of molybdenum reduction or Mo-blue production was bets modelled by Teissier followed by Luong, Aiba, Yano and Haldane. The calculated values of q_{max}, K_s and K_i for the Teissier model were 0.89 µmole Mo-blue per h, 5.84 mM and 32.23 mM, respectively (Halmi *et al.*, 2016b). The results indicate that the reduction rate of this bacterium is much slower than the strain Lbna but higher than *Serratia marcescens* Strain MIE2. Very few kinetic modelling studies have been carried out for metal biotransformation or bioreduction works. In most of such studies, the Haldane model was utilized to model reduction rate in the metals mercury [38], arsenate [39] and chromate [40].

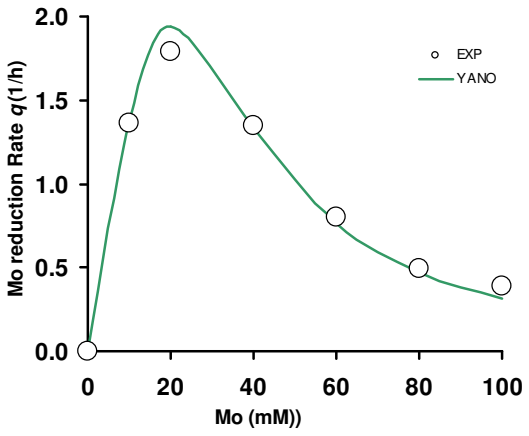

Fig. 2. Molybdenum reduction rate by *Pantoea* sp. strain HMY-P4 plotted against substrate (molybdate) concentrations as modelled according to the Yano model.

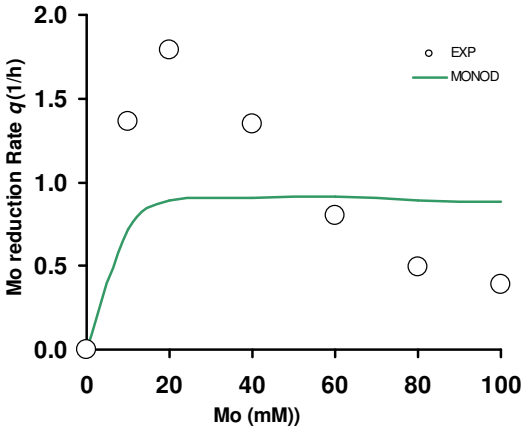

Fig. 3. Molybdenum reduction rate by *Pantoea* sp. strain HMY-P4 plotted against substrate (molybdate) concentrations as modelled according to the Monod model.

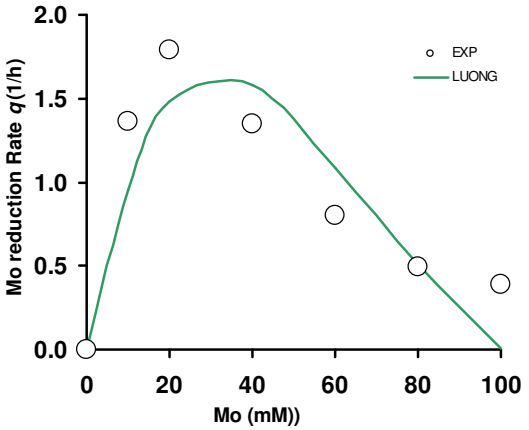

Fig. 4. Molybdenum reduction rate by *Pantoea* sp. strain HMY-P4 plotted against substrate (molybdate) concentrations as modelled according to the Luong model.

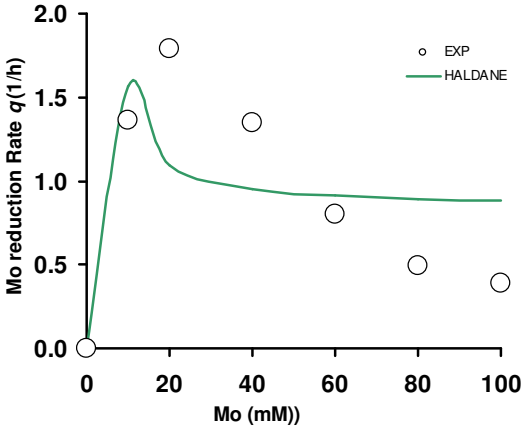

Fig. 5. Molybdenum reduction rate by *Pantoea* sp. strain HMY-P4 plotted against substrate (molybdate) concentrations as modelled according to the Haldane model.

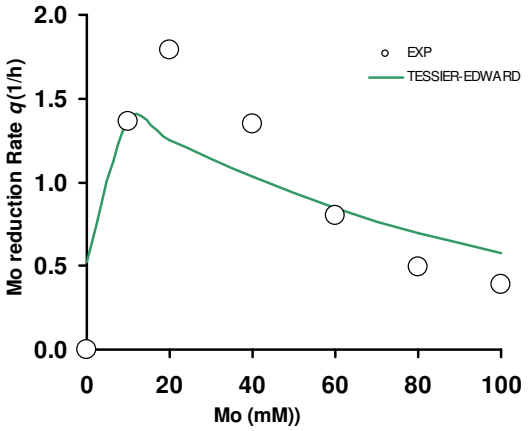

Fig. 6. Molybdenum reduction rate by *Pantoea* sp. strain HMY-P4 plotted against substrate (molybdate) concentrations as modelled according to the Teissier model.

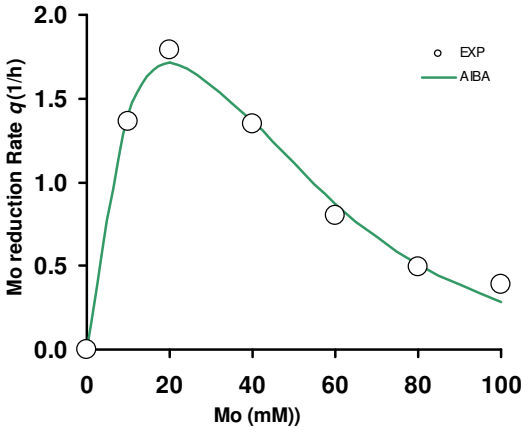

Fig. 7. Molybdenum reduction rate by *Pantoea* sp. strain HMY-P4 plotted against substrate (molybdate) concentrations as modelled according to the Aiba model.

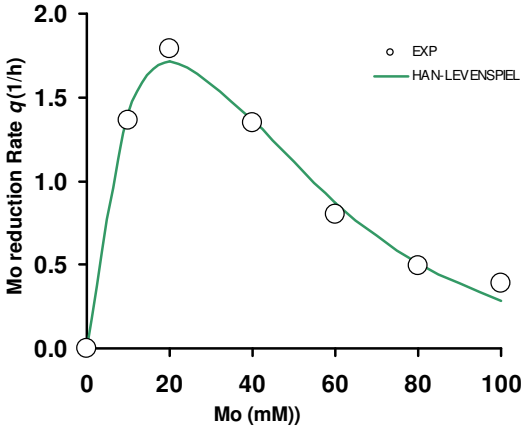

Fig. 7. Molybdenum reduction rate by *Pantoea* sp. strain HMY-P4 plotted against substrate (molybdate) concentrations as modelled according to the Hans-Levenspiel model.

Often biodegradation xenobiotics studies use substrates that impede the growth of the microbials or the biodegradation of the substrates because of their toxicity. Aromatic, halogenated and even essential processes of biotransformation that include metals such as mercury, chromium and molybdenum represent such inhibition examples [11,41,42]. The commonly used model to denote nontoxic substrate utilization rate, which is Monod will be unable to fit the rate curves and under this circumstances, other models such as Wayman and Tseng [43], Haldane, Luong, Han-Levenspiel, Andrews and Noack, and Webb should be used [44].

CONCLUSION

The kinetic modeling has shown that out of the seven models utilize to the effect of sodium molybdate as a substrate to Mo-blue production rate, only the Luong, Yano, Aiba and Han-Levenspiel appeared to be visually acceptable in their fittings of experimental data whilst other models appear to be inadequate. The best model as judged by statistical analysis is the Aiba model based on lowest values for RMSE, AICc, adjR^2, BF and AF values closest to 1.0 and the second best is the Yano model. The constants obtained from this modelling will be very important not only at the fundamental level but at the applied level especially when results from the laboratory need to be transformed to the field.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

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