

Modelling the Kinetics Molybdenum Reduction Rate by *Morganella* sp.

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ABSTRACT

Simulation of physical, chemical or biological data by mathematical approach can aid the researcher explain the physicochemical or biochemical phenomenon. Mathematical models can also be utilized to estimate or predict the possible actions, to replicate a prediction or retroactivity and to schedule better experiments. Bacteria are an emerging instrument for the bioremediation of the molybdenum pollutant in the world, molybdenum reduction into insoluble molybdenum blue. In this study, the molybdenum reduction rate by the bacterium *Morganella* sp. 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 value for the Teissier-Edward'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 7.77 (95% C.I., 4.41 to 19.95) $\mu\text{mol Mo-blue hr}^{-1}$, 26.63 (95% C.I., 12.82 to 40.44) mM and 51.39 (95% C.I., 23.67 to 79.10) mM, respectively. The true maximal reduction rate, which occurred when the slope of the curve is zero occurs at 36 mM molybdate concentration and a corresponding value of 1.85 $\mu\text{mol Mo-blue hr}^{-1}$. The model parameters obtained revealed that *Morganella* sp. have strong resistance to higher toxic molybdenum levels indicating the usefulness of this bacterium for bioremediation.

INTRODUCTION

Bioremediation is a method of removal by micro-organism of toxic heavy metals and molybdenum from the air. Molybdenum is a global source of pollution [1]. Microorganisms potentially detoxify molybdenum faster, easier and safer than conventional and physical approaches especially when soil contamination is the main target. Under this condition other methods are likely less efficient or expensive [2].

The use of more than 100 mg/L of molybdenum has a negative influence on mice testis (complete lack of libido and sterility). This is accompanied with alterations in the levels of the enzymes malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GPx) [3]. It therefore indicates, by altering the complex oxidative stress processes occurring in the testes, molybdenum can affect sperm quality. Rats that undergone molybdenum treatment with 12 mg/kg/day of tetrathiomolybdate up to sixty days exhibit significant decrease in their epididymal weight and sperm motility, count.

Furthermore, morphologicals along with histopathological effects in the epididymis and testes have been observed [4]. 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 [5]. Molybdenum is one of the essential trace elements that is necessary for more than 50 enzymes and acts as a micronutrient [6]. 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 [7–12]. 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 [13,14]. 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 [15,16]. Literature search shows that a Haldane-type inhibition is reported in several metal reduction kinetics studies such as mercury [17], arsenate [18] and chromate [19], while in the bacterial reduction of uranium, a Monod model is reported [20]. 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). In this study, a total of seven rate of bioreduction models will be utilized (**Table 1**).

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

Author	Degradation Rate	Author
Monod	$q_{\max} \frac{S}{K_s + S}$	[21]
Haldane	$q_{\max} \frac{S}{S + K_s + \frac{S^2}{K_i}}$	[22]
Teissier	$q_{\max} \left(1 - \exp\left(-\frac{S}{K_i}\right) - \exp\left(\frac{S}{K_i}\right) \right)$	[23]
Aiba-Edward	$q_{\max} \frac{S}{K_s + S} \exp\left(\frac{-S}{K_i}\right)$	[23,24]
Yano and Koga	$\frac{q_{\max} S}{S + K_s + \left(\frac{S^2}{K_i}\right) \left(1 + \frac{S}{K}\right)}$	[25]
Edward (Webb)	$q_{\max} \frac{S \left(1 + \frac{S}{K}\right)}{S + K_s + \left(\frac{S^2}{K_i}\right)}$	[23]
Luong	$q_{\max} \frac{S}{S + K_s} \left[1 - \left(\frac{S}{S_m}\right)^n \right]$	[16]

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)

MATERIALS AND METHOD

Bacterium and culture media preparation

All media preparations (solid and broth) were made according to the recipe of Shukor *et al.* [26] except otherwise stated. Solid medium was prepared by the addition of 18 g agar per L of medium. The Mo-reducing bacterium used in this study was previously isolated and identified as *Morganella* sp. [27].

Low phosphate-molybdate medium (LPM) and agar

A low phosphate medium allows the formation of Mo-blue whilst higher phosphate concentrations can inhibit reduction [28]. The medium was prepared (NH_4)₂SO₄, 3 g, MgSO₄·7H₂O, 0.5 g, NaCl, 5 g, Na₂MoO₄·2H₂O, 2.42g, Na₂HPO₄, 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 [29].

Modeling bioreduction kinetic experiment

The rate of the reduction of molybdenum to Mo-blue Molybdenum was carried out according a previous method [14]. 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).

Statistical discriminatory analysis

As the seven models have different parameters, the error function analysis utilized in this study incorporate penalty function for parameter. This include the following error function analyses;

The RMSE was calculated based on Eqn. 1. The lower number of parameters of the model is supposed to yield a smaller RMSE value [30].

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

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

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

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

Where,

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

RMS is Residual Mean Square

Another method based on information theory is the Akaike Information Criterion (AIC) [31]. The lowest value for AIC generally indicates favorable model. 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.[32]. The AICc is calculated for each data set for each model according to the following equation (Eqn. 4);

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

Where,

n is the number of data points,

p is the number of parameters.

Ross and McMeekin introduced the Accuracy Factor (AF) and Bias Factor (BF) calculated according to Eqns. 5 and 6 as suggested by Ross [33]. 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

Micro-organisms use mechanisms for detoxifying metal ions such as molybdenum, chrome, copper and mercury such as bioprecipitation, bioaccumulation and sequestration; efflux pumping and biosorption [34–37]. Microbial molybdate (Mo^{6+}) reduction was reported in the 19th century in *E. coli* by Capaldi and Proskauer [38]. However, the details on the reduction phenomenon were only being discovered during the last three decades by Campbell *et al.* in *E. coli* K12 [39]. The work of [40] on *Thiobacillus ferrooxidans* (now *Acidithiobacillus ferrooxidans*) then continued on *Enterobacter cloacae* strain 48 (EC 48) [41] and *Serratia marcescens* strain Dr.Y6 [42] and

recent one microbial-based molybdenum remediation which involves the reduction of molybdenum to a precipitable form, Mo-blue. Despite this, very few reduction kinetics studies have been poorly carried out.

The effect of different concentrations of molybdate to molybdenum reduction after 24 h of incubation shows that molybdenum blue production increases until 40 mM but starting to decrease at higher concentrations.

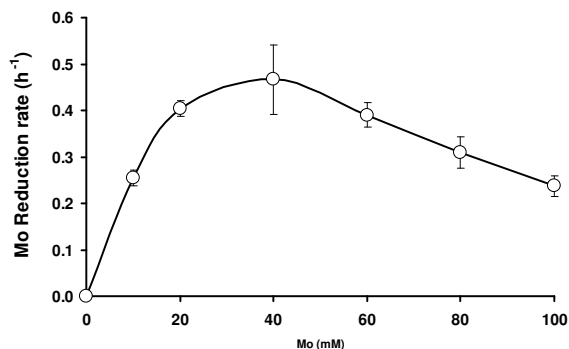


Fig. 1. Rate of Mo-blue production at various concentrations of molybdenum. Error bars represent mean \pm standard deviation (n=3).

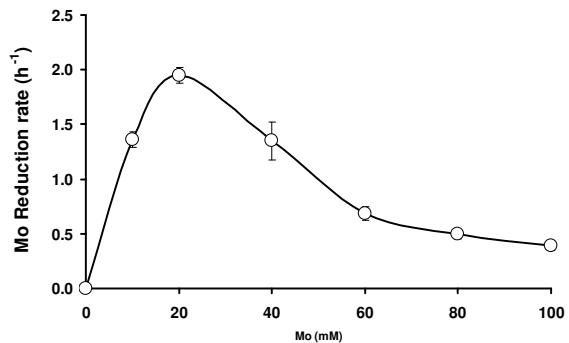


Fig. 1. The effect of sodium molybdate as a substrate to molybdenum reduction by the bacterium. Error bars represent mean \pm standard deviation (n=3).

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 7). 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, $\text{adj}R^2$, 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	adR2	AICc	BF	AF
Luong	4	n.a.	n.a.	n.a.	n.a.	n.a.
Yano	4	0.010	1.000	7	1.000	1.004
Tessier-Edward	3	0.035	0.996	-17	1.002	1.018
Aiba	3	0.036	0.995	-16	1.002	1.019
Haldane	3	0.400	0.224	17	1.026	1.210
Monod	2	0.357	0.418	1	1.026	1.210
Han and Levenspiel	5	0.051	0.986	n.a.	1.002	1.210

Note:

p no of parameters

adR² Adjusted Coefficient of determination

BF Bias factor

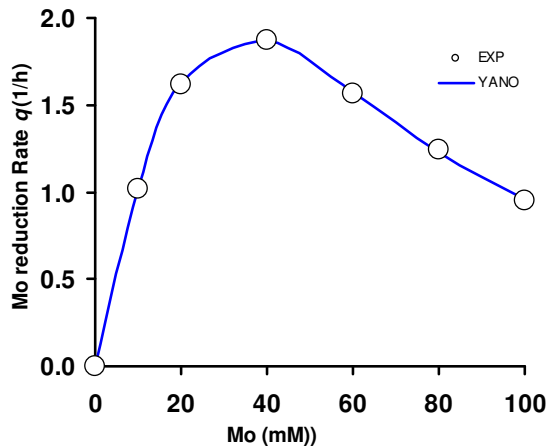
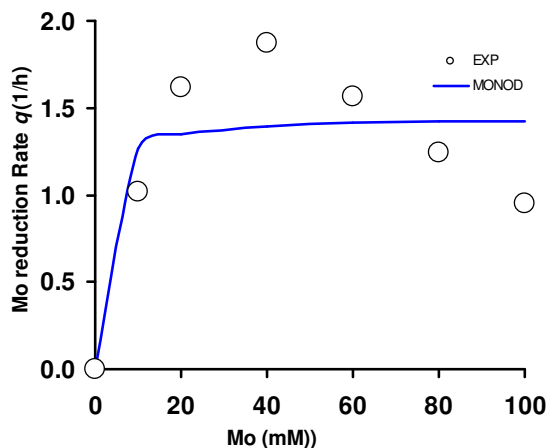
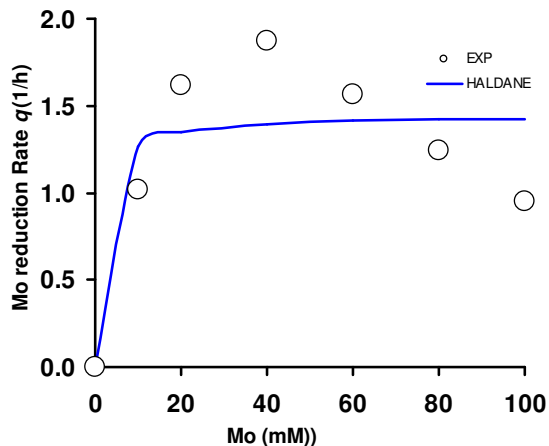
AF Accuracy factor

AICc Adjusted Akaike Information Criterion

The calculated value for the Teissier-Edward'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 7.77 (95% C.I., 4.41 to 19.95) $\mu\text{mole Mo-blue hr}^{-1}$, 26.63 (95% C.I., 12.82 to 40.44) mM and 51.39 (95% C.I., 23.67 to 79.10) mM, respectively. The true maximal reduction rate, which occurred when the slope of the curve is zero occurs at 36 mM molybdate concentration and a corresponding value of 1.85 $\mu\text{mole Mo-blue hr}^{-1}$ [43].

The Teissier or commonly also known as Tessier model has been used to model other microorganism rate processes. For example, it has been utilized in modelling asphaltene biodegradation using bacteria isolated from oil samples [44], modelling the biodegradation of Bisphenol A by the bacterium *Pseudomonas aeruginosa* PAb1 [45], removal of chromium (VI) by the bacterium *Bacillus subtilis* [46], modelling the production of microbial rennet by the fungus *Rhizopus chinensis* Saito BIOTECH 3273 using the substrate coconut paring cake [47], modelling the feed profile optimization of a batch fed alcoholic fermentation [48] and modelling the growth and biosynthetic kinetics of the production of the medium-chain-length poly-(3-hydroxyalkanoates) by the bacterium *Pseudomonas putida* [49].

This is the second time the Teissier-Edward model is found to best fit the Mo-blue production rate in Mo-reducing bacterium. The rate of molybdenum reduction or Mo-blue production. In the bacterium *Serratia marcescens* Strain MIE2 was best modelled by the Teissier model followed by Luong, Aiba, Yano and Haldane. The calculated values of q_{max} , K_s and K_i for the Teissier model are 0.89 $\mu\text{mole Mo-blue h}^{-1}$, 5.84 mM and 32.23 mM, respectively [14]. 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 $\mu\text{mole Mo-blue hr}^{-1}$, 115.8 mM, 57.83 mM and 1.405, respectively [50]. 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 [17], arsenate [19] and chromate [18].

**Fig. 2.** Molybdenum reduction rate by *Morganella* sp. plotted against substrate (molybdate) concentrations as modelled according to the Yano model.**Fig. 3.** Molybdenum reduction rate by *Morganella* sp. plotted against substrate (molybdate) concentrations as modelled according to the Monod model.**Fig. 4.** Molybdenum reduction rate by *Morganella* sp. plotted against substrate (molybdate) concentrations as modelled according to the Haldane model.

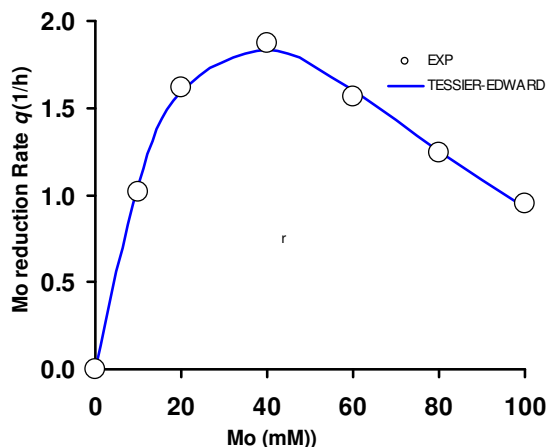


Fig. 5. Molybdenum reduction rate by *Morganella* sp. plotted against substrate (molybdate) concentrations as modelled according to the Teissier model.

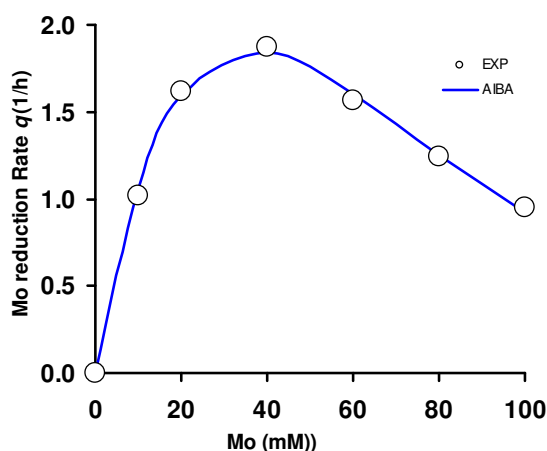


Fig. 6. Molybdenum reduction rate by *Morganella* sp. plotted against substrate (molybdate) concentrations as modelled according to the Aiba model.

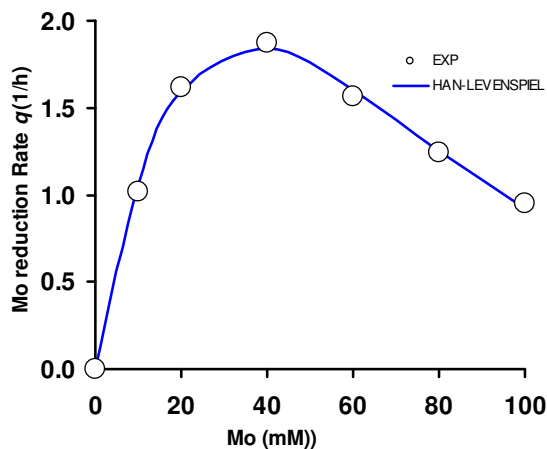


Fig. 7. Molybdenum reduction rate by *Morganella* sp. plotted against substrate (molybdate) concentrations as modelled according to the Hans-Levenspiel model.

Perhaps, studies on xenobiotics biodegradation utilize 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 [13,51,52]. 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 [53], Haldane, Luong, Han-Levenspiel, Andrews and Noack, and Webb should be used [54].

CONCLUSION

In conclusion, modeling kinetic has shown that of the seven models utilized to ascertain 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, $\text{adj}R^2$, 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.

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