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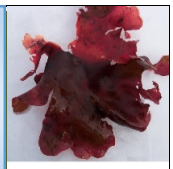

Modelling the Inhibition Kinetics of Molybdenum Reduction by the Molybdate-reducing *Enterobacter cloacae*

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ABSTRACT

The mathematical simulation of physical, chemical or biological data can assist a researcher in explaining the physicochemical or biological phenomenon by predicting or estimating 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 *Enterobacter cloacae* 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) µmole 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 µmole Mo-blue hr^{-1}. The fitting parameters of the Monod model prove a strong bacterial resistance to higher toxic molybdenum levels, making the bacterium useful tool for metals bioremediation work.

INTRODUCTION

In recent years, environmental bacterial remediation has attracted much attention as another exciting disruptive technology [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]. Molybdenum can be presented in the form of molybdenite, a byproduct of gold and copper mining. During past years, the contamination of several thousand acres of paddy land had occurred from episodic pollution cases due to the leakage from mining sites. The Mo concentration was reported to reach hundreds of ppm in Tokyo Bay, Japan [3] while in Tyrol Austria, hundreds acres of agricultural land had been contaminated by the pollution from industrial exhaust gas of which the earliest success of bioremediation recorded was achieved [4]. In Malaysia, the main and unnoticeable source of molybdenum pollution in Malaysia is via the indiscriminate release of lubricant oil wastes contained with 1 to 5 percent of

molybdenum to the environment resulting in high concentration of molybdenum in soils [1].

Many metal ions are required at trace concentrations while high level of metal ions is toxic at high concentrations. Microorganisms, employs a variety of methods to detoxify toxic metal ions and include methods such as efflux pumping, bioprecipitation, bioaccumulation, sequestration or chelation and biosorption [5–8].

The phenomenon or molybdate (Mo^{6+}) reduction to molybdenum blue is an old phenomenon where it was reported in the early of the 19th century in the bacterium *E. coli* [9]. It was Campbell *et al.* in the 80s that resuscitated the phenomenon by reporting it again in *E. coli* K12 [10]. The work of [11] on *Thiobacillus ferrooxidants* (now *Acidithiobacillus ferrooxidans*) then continued on *Enterobacter cloacae* strain 48 (EC 48) [12] not as a bioremediation agent but as a biomining candidate. Despite this, very few reports on its reduction kinetics studies have been reported. The only few reports are in *Bacillus* sp A.rzi and *Serratia* sp. MIE2, where mathematical modelling performed on the 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].

The predominant model in the literature is the Haldane-type inhibition, where it is reported in several metal reduction kinetics studies such as mercury [17], arsenate [18] and [19] chromate, 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**).

MATERIALS AND METHOD

Bacterium and culture media preparation

All media preparations (solid and broth) were made according to the recipe of Shukor *et al.* [21] 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 *Enterobacter cloacae* [22].

Low phosphate-molybdate medium (LPM) and agar

A low phosphate medium allows the formation of Mo-blue whilst higher phosphate concentrations can inhibit reduction [23]. 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} [24].

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

Author	Degradation Rate	Author
Monod	$q_{\max}\frac{S}{K_s+S}$	[25]
Haldane	$q_{\max}\frac{S}{S+K_s+\frac{S^2}{K_i}}$	[26]
Teissier	$q_{\max}\left(1-\exp\left(-\frac{S}{K_i}\right)-\exp\left(\frac{S}{K_s}\right)\right)$	[27]
Aiba-Edward	$q_{\max}\frac{S}{K_s+S}\exp\left(\frac{-S}{K_i}\right)$	[27,28]
Yano and Koga	$\frac{q_{\max}S}{S+K_s+\left(\frac{S^2}{K_1}\right)\left(1+\frac{S}{K}\right)}$	[29]
Edward (Webb)	$q_{\max}\frac{S\left(1+\frac{S}{K}\right)}{S+K_s+\left(\frac{S^2}{Ki}\right)}$	[27]
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)

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].

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

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

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

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

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**);

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

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 where by 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.

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

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

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

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 [4]. Molybdenum is one of the essential trace elements that is necessary for more than 50 enzymes and acts as a micronutrient [34]. 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 [35–40]. 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.

The effect of different concentrations of molybdate to molybdenum reduction after 24 h of incubation shows that molybdenum blue production appears not to be inhibited at extremely high concentrations. This is the first such observation, indicating that this bacterium is very hardy and resilient.

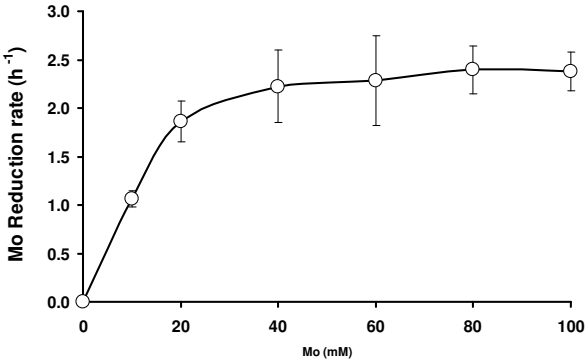

Fig. 1. Rate of Mo-blue production at various concentrations of molybdenum. Error bars represent mean ± standard deviation (n=3).

The data from the experimental values in batch studies was fitted to seven kinetic models using CurveExpert software (v1.6) to find the constants (**Figs. 2 to 7**). Of all the tested models, Luong model appeared inadequate. The best model as judged by statistical analysis is the Monod 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).

Model	p	RMSE	adR2	AICc	BF	AF
Luong	**4**	n.a.	n.a.	n.a.	n.a.	n.a.
Yano	**4**	0.106	0.979	41	1.006	1.031
Tessier-Edward	**3**	0.078	0.990	-6	1.006	1.027
Aiba	**3**	0.095	0.985	-3	1.006	1.032
Haldane	**3**	0.092	0.986	-3	1.006	1.031
Monod	**2**	0.122	0.976	-14	1.009	1.049
Han and Levenspiel	**5**	0.134	0.955	n.a.	1.006	1.049

Note:
p no of parameters
adR^2 Adjusted Coefficient of determination
BF Bias factor
AF Accuracy factor
AICc Adjusted Akaike Information Criterion
n.a. not available

In this work, the parameters for the molybdenum reduction kinetics are q_{max} and K_s, are maximum Mo-blue production rate (hr^{-1}) and half-saturation constant (mM). The calculated value for this model were 2.77 µmole Mo-blue hr^{-1} (95% C.I., 2.43 to 3.11) and 12.42 mM (95% C.I., 6.06 to 18.78), respectively.

The Monod model is often used to model the effect of nontoxic substrate to the rate of biological processes [41]. as for toxic substrates, rates at higher concentrations of substrates are often inhibitory and under this conditions, other substrate inhibition model such as Haldane, Teissier-Edwards, Yano, Aiba and Luong are more often observed [15,16,29,42]. The Monod model is observed for toxic substrate under mostly specific conditions, where the microorganisms are being immobilized in the forms of artificial substrate like gellan gum or polyacrylamide or biofilm [43–51].

The immobilization matrix and conditions form a kind of barrier to the entry of high concentration of toxicants into the cellular biomass and is likely the reason for the ability of the microorganism to be tolerant to higher concentration of toxicants. In this study, the bacterium is not immobilized and forms a very few instances where the bacterium itself is resilient through unknown defense processes.

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 µmole 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 µmole Mo-blue hr^{-1}, 115.8 mM, 57.83 mM and 1.405, respectively [52].

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]. In other similar works with xenobiotics, the Luong model has been fount to be the best model for several cases such as the degradation kinetics of *Bacillus cereus* grown on phenol [53], modelling of tributytin-resistant *Klebsiella* sp. FIRD 2 In cadmium media [54], growth inhibition kinetics of *Rhodotorula* sp. strain MBH23 (KCTC 11960BP) on acrylamide [55] and the inhibition kinetics of crude glycerol fermentation for 1,3-Dihydroxyacetone production [56].

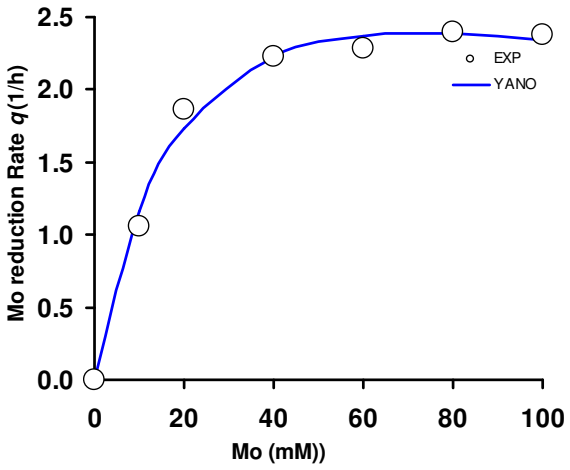

Fig. 2. Molybdenum reduction rate by *Enterobacter cloacae* plotted against substrate (molybdate) concentrations as modelled according to the Yano model.

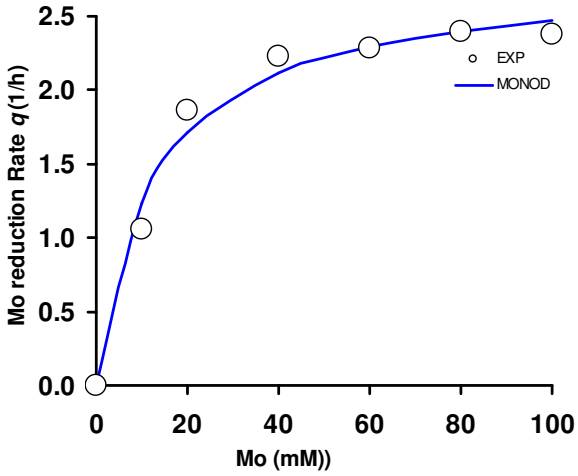

Fig. 3. Molybdenum reduction rate by *Enterobacter cloacae* plotted against substrate (molybdate) concentrations as modelled according to the Monod model.

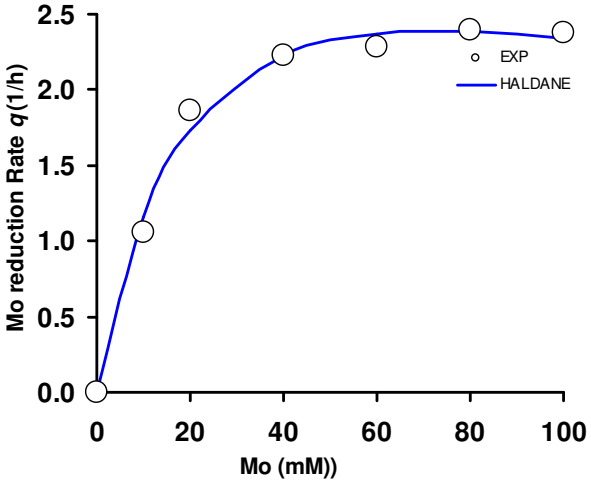

Fig. 4. Molybdenum reduction rate by *Enterobacter cloacae* plotted against substrate (molybdate) concentrations as modelled according to the Haldane model.

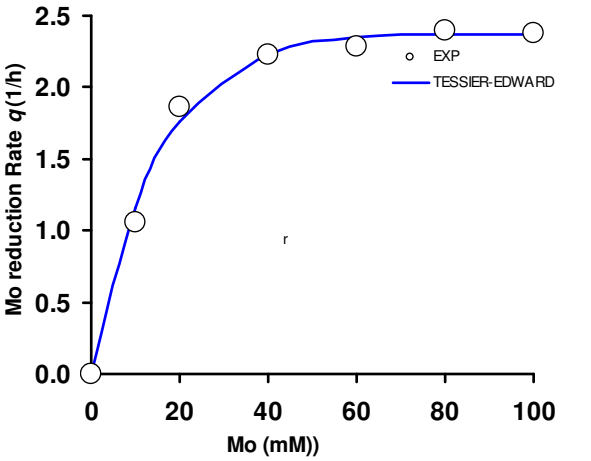

Fig. 5. Molybdenum reduction rate by *Enterobacter cloacae* plotted against substrate (molybdate) concentrations as modelled according to the Teissier model.

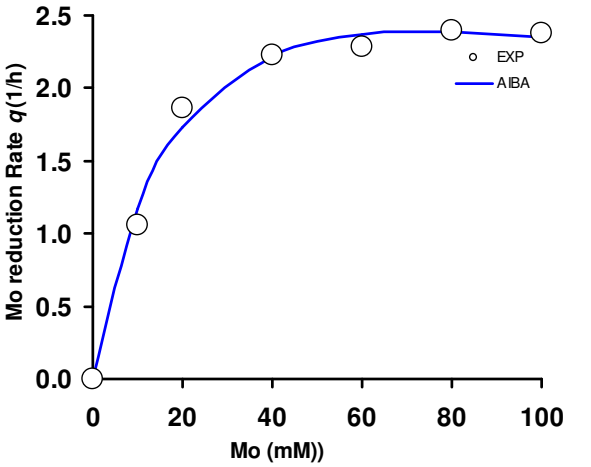

Fig. 6. Molybdenum reduction rate by *Enterobacter cloacae* plotted against substrate (molybdate) concentrations as modelled according to the Aiba model.

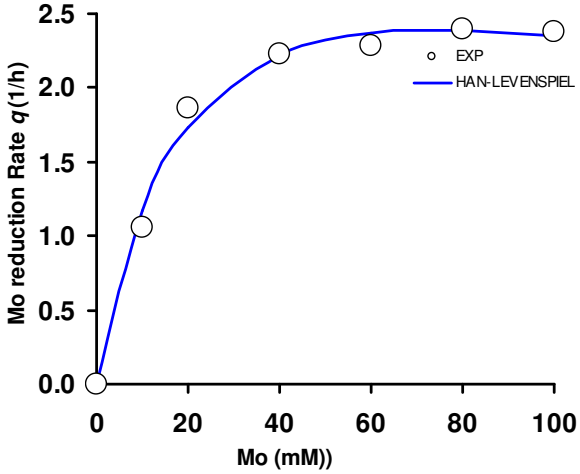

Fig. 7. Molybdenum reduction rate by *Enterobacter cloacae* plotted against substrate (molybdate) concentrations as modelled according to the Hans-Levenspiel model.

CONCLUSION

Conclusively, amongst the seven kinetic models used to monitor the effect of sodium molybdate as a substrate to Mo-blue production rate, only the Aiba, Han-Levenspiel, Luong and Yano seemed 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 gotten from this modelling exercise will be very vital not only at the fundamental level but could be applied in transforming laboratory findings to the field practice.

CONFLICT OF INTERESTS

The authors declare no conflict interest regarding this publication.

REFERENCE

1. Yakasai HM, Rahman MF, Yasid NA, Ahmad SA, Halmi MIE, Shukor MY. Elevated Molybdenum Concentrations in Soils Contaminated with Spent Oil Lubricant. J Environ Microbiol Toxicol. 2017 Dec 31;5(2):1–3.
2. Gafar AA, Shukor MY. Characterisation of an acrylamide-degrading bacterium and its degradation pathway. J Environ Microbiol Toxicol. 2018 Dec 31;6(2):29–33.
3. Davis GK. Molybdenum. In: Merian E, editor. Metals and their Compounds in the Environment, Occurrence, Analysis and Biological Relevance. New York: VCH Weinheim; 1991. p. 1089–100.
4. Neunhäuserer C, Berreck M, Insam H. Remediation of soils contaminated with molybdenum using soil amendments and phytoremediation. Water Air Soil Pollut. 2001;128(1–2):85–96.
5. Halmi MIE, Zuhainis SW, Yusof MT, Shaharuddin NA, Helmi W, Shukor Y, et al. Hexavalent molybdenum reduction to Mo-blue by a sodium-dodecyl-sulfate- degrading *Klebsiella oxytoca* strain dry14. BioMed Res Int. 2013;2013(December 2013):1–8.
6. Lloyd JR. Microbial reduction of metals and radionuclides. FEMS Microbiol Rev. 2003;27(2–3):411–25.
7. Othman AR, Bakar NA, Halmi MIE, Johari WLW, Ahmad SA, Jirangon H, et al. Kinetics of molybdenum reduction to molybdenum blue by *Bacillus* sp. strain A.rzi. BioMed Res Int. 2013;2013:1–9.

8. Shukor MY, Rahman MF, Shamaan NA, Syed MS. Reduction of molybdate to molybdenum blue by *Enterobacter* sp. strain Dr.Y13. J Basic Microbiol. 2009;49(SUPPL. 1):43–54.
9. Levine VE. The reducing properties of microorganisms with special reference to selenium compounds. J Bacteriol. 1924;X:217–62.
10. Campbell AM, Del Campillo-Campbell A, Villaret DB. Molybdate reduction by *Escherichia coli* K-12 and its chl mutants. Proc Natl Acad Sci U S A. 1985;82(1):227–31.
11. Sugio T, Tsujita Y, Katagiri T, Inagaki K, Tano T. Reduction of Mo6+ with Elemental Sulfur by Thiobacillus ferrooxidans. J Bacteriol. 1988;170(12):5956–9.
12. Ghani B, Takai M, Hisham NZ, Kishimoto N, Ismail AKM, Tano T, et al. Isolation and Characterization of a Mo6+-Reducing Bacterium. Appl Environ Microbiol. 1993;59(4):1176–80.
13. Othman AR, Bakar NA, Halmi MIE, Johari WLW, Ahmad SA, Jirangon H, et al. Kinetics of molybdenum reduction to molybdenum blue by *Bacillus* sp. strain A.rzi. BioMed Res Int. 2013;2013:Article number 371058.
14. Halmi MIE, Abdullah SRS, Johari WLW, Ali MSM, Shaharuddin NA, Khalid A, et al. Modelling the kinetics of hexavalent molybdenum (Mo6+) reduction by the Serratia sp. strain MIE2 in batch culture. Rendiconti Lincei. 2016 Dec 1;27(4):653–63.
15. Teissier G. Growth of bacterial populations and the available substrate concentration. Rev Sci Instrum. 1942;3208:209–14.
16. Luong JHT. Generalization of Monod kinetics for analysis of growth data with substrate inhibition. Biotechnol Bioeng. 1987;29(2):242–8.
17. Gluszcz P, Petera J, Ledakowicz S. Mathematical modeling of the integrated process of mercury bioremediation in the industrial bioreactor. Bioprocess Biosyst Eng. 2011;34(3):275–85.
18. Soda SO, Yamamura S, Zhou H, Ike M, Fujita M. Reduction kinetics of As (V) to As (III) by a dissimilatory arsenate-reducing bacterium, Bacillus sp. SF-1. Biotechnol Bioeng. 2006;93(4):812–5.
19. Sukumar M. Reduction of hexavalent chromium by *Rhizopus oryzae*. Afr J Environ Sci Technol. 2010;4(7):412–8.
20. Truex MJ, Peyton BM, Valentine NB, Gorby YA. Kinetics of U(VI) reduction by a dissimilatory Fe(III)-reducing bacterium under non-growth conditions. Biotechnol Bioeng. 1997 Aug 5;55(3):490–6.
21. Maarof MZ, Shukor MY, Mohamad O, Karamba KI, Halmi MIE, Rahman MFA, et al. Isolation and Characterization of a Molybdenum-reducing *Bacillus amyloliquefaciens* strain KIK-12 in Soils from Nigeria with the Ability to grow on SDS. J Environ Microbiol Toxicol. 2018 Jul 31;6(1):13–20.
22. Kabir ZM, Gafasa MA, Kabara HT, Ibrahim SS, Babandi A, M. Ya'u, et al. Isolation and Characterization of Molybdate-reducing *Enterobacter cloacae* from Agricultural Soil in Gwale LGA Kano State, Nigeria. J Environ Microbiol Toxicol. 2019 Jul 31;7(1):1–6.
23. Shukor MY, Lee CH, Omar I, Karim MIA, Syed MA, Shamaan NA. Isolation and characterization of a molybdenum-reducing enzyme in *Enterobacter cloacae* strain 48. Pertanika J Sci Technol. 2003;11(2):261–72.
24. Shukor MY, Shamaan NA, Syed MA, Lee CH, Karim MIA. Characterization and quantification of molybdenum blue production in *Enterobacter cloacae* strain 48 using 12-molybdophosphate as the reference compound. Asia-Pac J Mol Biol Biotechnol. 2000;8(2):167–72.
25. Monod J. The Growth of Bacterial Cultures. Annu Rev Microbiol. 1949;3(1):371–94.
26. Andrews JF. A mathematical model for the continuous culture of microorganisms utilizing inhibitory substrates. Biotechnol Bioeng. 1968 Nov 1;10(6):707–23.
27. Edwards VH. The influence of high substrate concentrations on microbial kinetics. Biotechnol Bioeng. 1970;12(5):679–712.
28. Aiba S, Shoda M, Nagatani M. Kinetics of product inhibition in alcohol fermentation. Biotechnol Bioeng. 1968 Nov 1;10(6):845–64.
29. Yano T, Koga S. Dynamic behavior of the chemostat subject to substrate inhibition. Biotechnol Bioeng. 1969 Mar 1;11(2):139–53.
30. Motulsky HJ, Ransnas LA. Fitting curves to data using nonlinear regression: a practical and nonmathematical review. FASEB J Off Publ Fed Am Soc Exp Biol. 1987;1(5):365–74.
31. Akaike H. New look at the statistical model identification. IEEE Trans Autom Control. 1974;AC-19(6):716–23.
32. Burnham KP, Anderson DR. Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach. Springer Science & Business Media; 2002. 528 p.
33. Ross T, McMeekin TA. Predictive microbiology. Int J Food Microbiol. 1994;23(3–4):241–64.
34. Bakar NA, Othman AR, Shukor MY. Heavy metals detection from contaminated river using molybdenum reducing enzyme. J Kejuruter. 2019 Oct;31(2):303–8.
35. Halmi MIE, Zuhainis SW, Yusof MT, Shaharuddin NA, Helmi W, Shukor Y, et al. Hexavalent molybdenum reduction to Mo-blue by a sodium-dodecyl-sulfate- degrading *Klebsiella oxytoca* strain DRY14. BioMed Res Int. 2013;2013:Article number 384541.
36. Masdor N, Abd Shukor MS, Khan A, Bin Halmi MIE, Abdullah SRS, Shamaan NA, et al. Isolation and characterization of a molybdenum-reducing and SDS- degrading *Klebsiella oxytoca* strain Aft-7 and its bioremediation application in the environment. Biodiversitas. 2015;16(2):238–46.
37. Khayat ME, Rahman MFA, Shukor MS, Ahmad SA, Shamaan NA, Shukor MY. Characterization of a molybdenum-reducing *Bacillus* sp. strain khayat with the ability to grow on SDS and diesel. Rendiconti Lincei. 2016;Article in Press.
38. Mansur R, Gusmanizar N, Roslan MAH, Ahmad SA, Shukor MY. Isolation and characterisation of a molybdenum-reducing and Metanil yellow dye-decolourising *Bacillus* sp. strain Neni-10 in soils from West Sumatera, Indonesia. Trop Life Sci Res. 2017 Jan;28(1):69–90.
39. Rahman MFA, Yasid NA, Ahmad SA, Shamaan NA, Shukor MY. Characterization of molybdenum-reduction by an acrylamide-degrading Antarctic bacterium. In 10-3 Midori-cho, Tachikawa, Tokyo, Japan: National Institute of Polar Research (NIPR); 2018. Available from: http://id.nii.ac.jp/1291/00015258/
40. Manogaran M, Ahmad SA, Yasid NA, Yakasai HM, Shukor MY. Characterisation of the simultaneous molybdenum reduction and glyphosate degradation by *Burkholderia vietnamiensis* AQ5-12 and *Burkholderia* sp. AQ5-13. 3 Biotech. 2018 Feb 7;8(2):117.
41. Monod J. The Growth of Bacterial Cultures. Annu Rev Microbiol. 1949;3(1):371–94.
42. Haldane JBS. Enzymes,. Longmans, Green and Co. London; 1930.
43. Karamanev DG, Samson R. High-rate biodegradation of pentachlorophenol by biofilm developed in the immobilized soil bioreactor. Environ Sci Technol. 1998 Apr 1;32(7):994–9.
44. González G, Herrera G, García MaT, Peña M. Biodegradation of phenolic industrial wastewater in a fluidized bed bioreactor with immobilized cells of *Pseudomonas putida*. Bioresour Technol. 2001 Nov 1;80(2):137–42.
45. Kermanshahi pour A, Karamanev D, Margaritis A. Kinetic modeling of the biodegradation of the aqueous p-xylene in the immobilized soil bioreactor. Biochem Eng J. 2006 Jan 1;27(3):204–11.
46. Chen Y-M, Lin T-F, Huang C, Lin J-C, Hsieh F-M. Degradation of phenol and TCE using suspended and chitosan-bead immobilized *Pseudomonas putida*. J Hazard Mater. 2007 Sep 30;148(3):660–70.
47. Robledo-Ortíz JR, Ramírez-Arreola DE, Pérez-Fonseca AA, Gómez C, González-Reynoso O, Ramos-Quirarte J, et al. Benzene, toluene, and o-xylene degradation by free and immobilized *P. putida* F1 of postconsumer agave-fiber/polymer foamed composites. Int Biodeterior Biodegrad. 2011 Jun 1;65(3):539–46.
48. Begum SS, Radha KV. Biodegradation kinetic studies on phenol in internal draft tube (inverse fluidized bed) biofilm reactor using *Pseudomonas fluorescens*: Performance evaluation of biofilm and biomass characteristics. Bioremediation J. 2013;17(4):264–77.
49. Surkatti R, El-Naas MH. Biological treatment of wastewater contaminated with p-cresol using *Pseudomonas putida* immobilized in polyvinyl alcohol (PVA) gel. J Water Process Eng. 2014 Apr 1;1:84–90.
50. Tan S, Chen X, Cui C, Hou Y, Li W, You H. Biodegradation of saline phenolic wastewater in a biological contact oxidation reactor with immobilized cells of *Oceanimonas* sp. Biotechnol Lett. 2017 Jan 1;39(1):91–6.
51. Abu Talha M, Goswami M, Giri BS, Sharma A, Rai BN, Singh RS. Bioremediation of Congo red dye in immobilized batch and

continuous packed bed bioreactor by *Brevibacillus parabrevis* using coconut shell bio-char. Bioresour Technol. 2018 Mar 1;252:37–43.

52. Halmi MIE, Ahmad SA, Syed MA, Shamaan NA, Shukor MY. Mathematical modelling of the molybdenum reduction kinetics in *Bacillus pumilus* strain Lbna. Bull Environ Sci Manag. 2014;2(1):24–9.
53. Halmi MIE, Shukor MS, Johari WLW, Shukor MY. Mathematical modelling of the degradation kinetics of *Bacillus cereus* grown on phenol. J Environ Bioremediation Toxicol. 2014;2(1):1–5.
54. Abdussamad A, Abdullahi M, Shehu D, Murtala Y, Abba B, Abubakar ST, et al. Modelling Growth Kinetics of *Klebsiella* sp. FIRD 2 on TBT-Resistant Containing Lead. J Appl Sci Environ Manag. 2017;21(6):1085–91.
55. Othman AR, Rahim MBHA. Modelling the Growth Inhibition Kinetics of *Rhodotorula* sp. strain MBH23 (KCTC 11960BP) on Acrylamide. Bioremediation Sci Technol Res. 2019 Dec 28;7(2):20–5.
56. Dikshit PK, Padhi SK, Moholkar VS. Process optimization and analysis of product inhibition kinetics of crude glycerol fermentation for 1,3-Dihydroxyacetone production. Bioresour Technol. 2017 Nov 1;244:362–70.