HIBISCUS

BULLETIN OF ENVIRONMENTAL SCIENCE AND MANAGEMENT

Website: http://journal.hibiscuspublisher.com

Mathematical modelling of the molybdenum reduction kinetics in *Bacillus pumilus* strain Lbna

Halmi, M.I.E[1], Ahmad, S.A.[1], Syed, M.A.[1], Shamaan, N.A.[2] and Shukor, M.Y.[1]

[1]Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, University Putra Malaysia, UPM 43400 Serdang, Selangor, Malaysia.
[2]Faculty of Medicine and Health Sciences, Universiti Sains Islam Malaysia, 13th Floor, Menara B, Persiaran MPAJ, Jalan Pandan Utama, Pandan Indah, 55100 Kuala Lumpur, Malaysia.
Corresponding author; Shukor, M.Y., E-mail: yunus.upm@gmail.com

History
Received: 24 April 2014
Received in revised form: 16 Mei 2014
Accepted: 6 July 2014
Available online: 25 July 2014

Keyword
Reduction kinetics;
Molybdenum;
Molybdenum Blue;
Bacillus;
Luong

Abstract

Molybdenum, an emerging pollutant, has just recently been demonstrated to be toxic to spermatogenesis in several animal model systems. Hence its removal and studies on the kinetics of removal is very important at a biotechnological point of view. In this work we study the reduction kinetics of a molybdenum-reducing bacterium using kinetic models such as Haldane, Monod, Teissier, Andrews and Noack, Hinshelwood, Moser, Aiba, Webb (Edward), Yano and Koga, Han and Levenspiel and Luong and evaluated the accuracy of the fitted model using statistical analysis such as Root Mean Square (RMSE), adjusted Coefficient of Determination (R^2), corrected Akaike Information Criterion (AICc), Bias Factor, Accuracy Factor and F-test. Statistical analysis shows that the best model was Luong with low values for RMSE and AICc, highest adjusted R^2 values, F-test and with Bias Factor and Accuracy Factor nearest to unity (1.0). The calculated value for the Luong's constants q_{max}, K_s, S_m, and n were 27.303 hr^{-1}, 115.8 mM, 57.83 mM and 1.405, respectively. The true q_{max} where the gradient for the slope is zero was approximately 3.38 h^{-1} at 30 mM molybdenum. 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 is very toxic to ruminants with levels as low as several parts per million causing scouring and even deaths (1,2). Recently, it was discovered that molybdenum shows its toxicity by inhibiting spermatogenesis in catfish and mice at levels as low as several parts per million (3–6). This new findings would increase molybdenum exposure as a toxic heavy metals similar to chromate and would increase the number of works on its removal from soil and water bodies. In the past decades researchers have focused on bioremediation as an environmental friendly and low cost method to solve this problem. Bioremediation is one of the ways to remove toxic metals from the environment (7). A variety of molybdenum reducing bacteria has been reported with all of them required a semi-aerobic condition for maximal production of molybdenum blue (8–18). One of the least studied area in molybdenum reduction to molybdenum blue (Mo-blue) is reduction kinetics. Only one study has been carried out in *Bacillus* sp. strain A.rzi and the best model was Luong (11). In most other metal reduction kinetic studies, the Haldane model is most often used to model reduction kinetics (19,20). This is despite the fact, that for a single substrate-inhibiting compound such as phenol, several other models have been demonstrated to be more accurate. For instance, aside from the predominantly reported Haldane model (21), several other different models have been found to be optimal such as Luong (22,23) and Edward (24). Hence, the use of extensive models available could replace the Haldane in some circumstances. Without actually fitting these other models to the available growth or degradation rate data and proper statistical evaluation, the exclusive use of the Haldane model should not be used liberally.

Hence, the objective of this work is to evaluate which model could be used in molybdenum reduction kinetics with extensive statistical analysis support. The data could be used for further more comprehensive secondary modelling. 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 degradation 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	$q_{\max}\frac{S}{K_s+S}\exp(-KP)$	(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)
Han and Levenspiel	$q_{\max}\left[1-\left(\frac{S}{S_m}\right)\right]^n\left[\frac{S}{S+K_s\left(1-\frac{S}{S_m}\right)^m}\right]$	(30)
Luong	$q_{\max}\frac{S}{S+K_s}\left[1-\left(\frac{S}{S_m}\right)^n\right]$	(31)

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)

MATERIAL AND METHODS

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 (32).

Isolation and maintenance of Molybdate-Reducing Bacterium

Bacillus pumilus strain Lbna was previously isolated, identified and characterized by Abo-Shakeer et al. (8). Growth and maintenance was carried out on solid agar in low phosphate media (pH 7.0) containing (2.9 mM phosphate), glucose (1%), $(NH_4)_2SO_4$ (0.3%), $MgSO_4.7H_2O$ (0.05%), NaCl (0.5%), yeast extract (0.0.5%), $Na_2MoO_4.2H_2O$ (0.242%) and Na_2HPO_4 (0.05%) (8). Glucose was autoclaved separately. Growth in liquid media uses the same media as in the solid media above. The production of molybdenum blue from the media was measured at 865 nm. Quantification of Mo-blue produced was carried out according to the method of Shukor et al. (33).

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 (8–18). Hence, only the reduction kinetics was studied. In this work molybdenum reduction kinetics is represented as Mo-blue production rate (μmole Mo-blue/h). The Mo-blue production rate is calculated based on the natural logarithm of the linear portion of the Mo-blue production against time.

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), corrected AIC (Akaike Information Criterion) and F-test (34).

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.

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

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 according to the formula where RMS is Residual Mean Square and S_y^2 is the total variance of the y-variable (34).

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

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

AIC provides a means for model selection through measuring the relative quality of a given statistical model for a given set of experimental data (35).

It 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 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 (36).

The F-test is a statistic test used to find the most significant model between available predicted curve-fitting models. The analysis procedure includes selecting the model with the smallest RSS among all the models with the same or different number of fitting parameters followed by comparing the relative value of the F-ratio. In the event the F-ratio of the two models surpasses the upper quartile, the better complicated model is accepted as statistically significant (36). Equation 5 is for models with same number of parameters while Equation 6 is for models with different number of parameters;

$$F = \frac{SS_1}{SS_2} \quad (5)$$

$$F = \frac{(SS_1 - SS_2)/(df_2 - df_1)}{SS_2 / df_2} \quad (6)$$

Accuracy Factor (AF) and Bias Factor (BF) to test for the goodness-of-fit of the models as suggested by Ross (37) were also used. The Bias Factor equal to1 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 > 1indicates a fail-safe model. The Accuracy Factor is always ≥ 1, and higher AF values indicate less precise prediction.

$$\text{Bias factor} = 10^{\left(\sum_{i=1}^{n}\log\frac{(Pd_i/Ob_i)}{n}\right)} \quad (7)$$

$$\text{Accuracyfactor} = 10^{\left(\sum_{i=1}^{n}\log\frac{|(Pd_i/Ob_i)|}{n}\right)} \quad (8)$$

RESULTS AND DISCUSSION

The results of the curve fitting are shown in Figures 2 to 5. Models such as Haldane, Teissier, Hinshelwood, Andrews and Noack, and Han and Levenspiel failed to fit the experimental data and were omitted. All of the other models tested with the exception of the Monod model gave reasonably good fitting based on visual observation.

Figure. 2. Fitting experimental data with the Yano model.

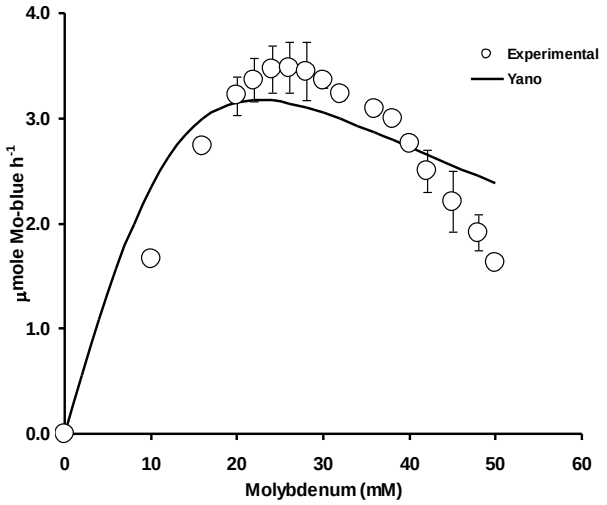


Figure. 3. Fitting experimental data with the Luong model.

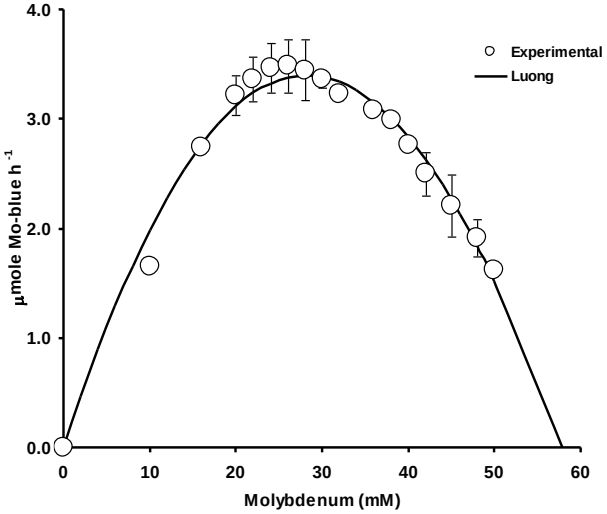


Figure. 4. Fitting experimental data with the Webb model.

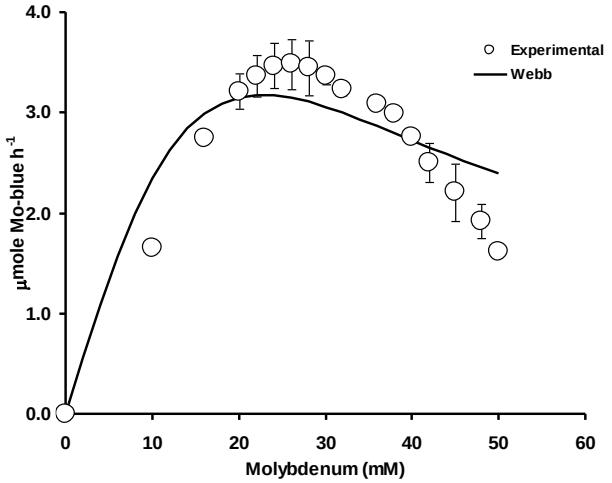
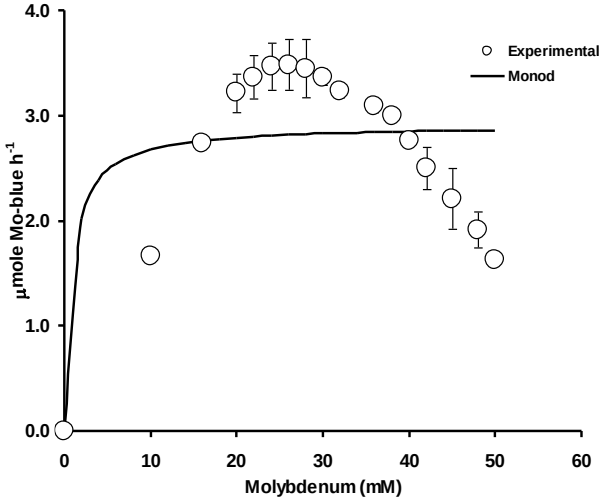


Figure. 5. Fitting experimental data with the Monod model.



The accuracy and statistical analysis of the four kinetic models used shows that the best model was Luong with low values for RMSE and AICc, highest adjusted R^2 values, F-test and with Bias Factor and Accuracy Factor nearest to unity (1.0) (Table 2). The calculated value for the Luong's constants maximal reduction rate, half saturation constant for maximal reduction, maximal concentration of substrate tolerated and curve parameter that defines the steepness of the growth rate decline from the maximum rate symbolized by q_{max}, K_s, S_m, and n were 27.303 hr^{-1}, 115.8 mM, 57.83 mM and 1.405, respectively. In this case even though the model predicted that the highest concentration that could inhibit rate of molybdenum reduction is 57.83, the value of the K_s was much higher than the value of the S_m meaning more data should be obtained to give more accurate modelling information.

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 (Luong) the value was approximately 3.38 h^{-1} at 30 mM molybdenum (Figure 3). The equation for the Luong using the values obtained from the fitting is as follows;

$$q=3.38\frac{S}{S+115.8}\left[1-\left(\frac{S}{57.83}\right)^{1.405}\right] \quad (9)$$

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 (11,38,39) and hence it can be deducted 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 (1976) (40). 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 (41). 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 (11,22,23) including this one. A central attraction of the Luong model is its ability to successful predicting the value of S_m, the maximum substrate concentration above which growth is completely inhibited.

Most studies on the reduction kinetics of heavy metals such as mercury (42), arsenate (20) and chromate (19) 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 (11,19,20,42,43), the Luong model allows for the determination of the critical concentration of substrate that could completely inhibited production of product (41) as evident from this work. This is the first time existing models of kinetic studies being applied to model Mo-blue production in a bacterium.

Table 2. Statistical analysis of kinetic models.

Model	Parameter	SSE	MSE	RMSE	R2	adR2	AICc	BF	AF
Luong	**4**	0.2199	0.0183	0.1354	0.983	0.977	-44.59	1.0030	1.0226
Webb	**4**	2.0408	0.1701	0.4124	0.771	0.688	-8.95	1.0204	1.0952
Yano	**4**	2.0452	0.1704	0.4128	0.771	0.687	-8.91	1.0199	1.0949
Monod	**2**	5.8965	0.4212	0.6490	0.210	0.088	-3.97	1.0104	1.1396

Note:
SSE Sums of Squared Errors
RMSE Root Mean Squared Error
R^2 Coefficient of Determination
adR^2 Adjusted Coefficient of Determination
AICc Corrected Akaike Information Criterion
BF Bias Factor
AF Accuracy Factor

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 Luong model is the best model in fitting the reduction kinetics data from *Bacillus pumilus* strain Lbna similar to the only Mo-reducing bacterium with published kinetics data. Whether the Luong model is generally applicable to other Mo-reducing bacterium is an important future works.

REFERENCES

[1] Greenwood NN, Earnshaw A. Chemistry of the elements. Pergammon Press, Oxford; 1984.
[2] Stojek, M.M. The concentration of molybdenum and copper in rocks, soils and plants in the area of Jabłonki (Eastern Beskids Mts.) / Zawartość molibdenu i miedzi w skałach, glebach i roślinach w okolicy Jabłonek (Beskidy Wschodnie). Ochr Śr Zasobów Nat - Environ Prot Nat Resour. 2013;24(3):13–7.
[3] Yamaguchi S, Miura C, Ito A, Agusa T, Iwata H, Tanabe S, et al. Effects of lead, molybdenum, rubidium, arsenic and organochlorines on spermatogenesis in fish: Monitoring at Mekong Delta area and in vitro experiment. Aquat Toxicol. 2007;83(1):43–51.
[4] Zhai X-W, Zhang Y-L, Qi Q, Bai Y, Chen X-L, Jin L-J, et al. Effects of molybdenum on sperm quality and testis oxidative stress. Syst Biol Reprod Med. 2013;59(5):251–5.
[5] Bi C-M, Zhang Y-L, Liu F-J, Zhou T-Z, Yang Z-J, Gao S-Y, et al. The effect of molybdenum on the in vitro development of mouse preimplantation embryos. Syst Biol Reprod Med. 2013;59(2):69–73.
[6] Zhang Y-L, Liu F-J, Chen X-L, Zhang Z-Q, Shu R-Z, Yu X-L, et al. Dual effects of molybdenum on mouse oocyte quality and ovarian oxidative stress. Syst Biol Reprod Med. 2013;59(6):312–8.
[7] Ahmad SA, Shamaan NA, Arif NM, Koon GB, Shukor MYA, Syed MA. Enhanced phenol degradation by immobilized Acinetobacter sp. strain AQ5NOL 1. World J Microbiol Biotechnol. 2012;28(1):347–52.
[8] Abo-Shakeer, L.K.A., Ahmad, S.A., Shukor, M.Y., Shamaan NA, Syed, M.A. Isolation and characterization of a molybdenum-reducing Bacillus pumilus strain lbna. J Environ Microbiol Toxicol. 2013;1(1):9–14.
[9] Ahmad SA, Shukor MY, Shamaan NA, Mac Cormack WP, Syed MA. Molybdate reduction to molybdenum blue by an antarctic bacterium. BioMed Res Int. 2013;2013.
[10] Lim HK, Syed MA, Shukor MY. Reduction of molybdate to molybdenum blue by Klebsiella sp. strain hkeem. J Basic Microbiol. 2012;52(3):296–305.
[11] 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.
[12] Shukor MY, Ahmad SA, Nadzir MMM, Abdullah MP, Shamaan NA, Syed MA. Molybdate reduction by Pseudomonas sp. strain DRY2. J Appl Microbiol. 2010;108(6):2050–8.
[13] Shukor MY, Habib SHM, Rahman MFA, Jirangon H, Abdullah MPA, Shamaan NA, et al. Hexavalent molybdenum reduction to molybdenum blue by S. Marcescens strain Dr. Y6. Appl Biochem Biotechnol. 2008;149(1):33–43.
[14] Shukor MY, Halmi MIE, Rahman MFA, Shamaan NA, Syed MA. Molybdenum reduction to molybdenum blue in Serratia sp. strain DRY5 is catalyzed by a novel molybdenum-reducing enzyme. BioMed Res Int. 2014;2014.
[15] 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):S43–S54.
[16] Shukor MY, Rahman MF, Suhaili Z, Shamaan NA, Syed MA. Bacterial reduction of hexavalent molybdenum to molybdenum blue. World J Microbiol Biotechnol. 2009;25(7):1225–34.
[17] Shukor MY, Rahman MF, Suhaili Z, Shamaan NA, Syed MA. Hexavalent molybdenum reduction to Mo-blue by Acinetobacter calcoaceticus. Folia Microbiol (Praha). 2010;55(2):137–43.
[18] Yunus SM, Hamim HM, Anas OM, Aripin SN, Arif SM. Mo (VI) reduction to molybdenum blue by Serratia marcescens strain Dr. Y9. Pol J Microbiol. 2009;58(2):141–7.
[19] 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.
[20] Sukumar M. reduction of hexavalent chromium by Rhizopus Oryzae. Afr J Environ Sci Technol. 2010;4(7):412–8.
[21] Arif NM, Ahmad SA, Syed MA, Shukor MY. Isolation and characterization of a phenol-degrading Rhodococcus sp. strain AQ5NOL 2 KCTC 11961BP. J Basic Microbiol. 2013;53(1):9–19.

22] Hamitouche A-E, Bendjama Z, Amrane A, Kaouah F, Hamane D. Relevance of the Luong model to describe the biodegradation of phenol by mixed culture in a batch reactor. Ann Microbiol. 2012;62(2):581–6.
[23] Nickzad A, Mogharei A, Monazzami A, Jamshidian H, Vahabzadeh F. Biodegradation of phenol by Ralstonia eutropha in a Kissiris-immobilized cell bioreactor. Water Environ Res. 2012;84(8):626–34.
[24] Saravanan P, Pakshirajan K, Saha P. Kinetics of phenol and m-cresol biodegradation by an indigenous mixed microbial culture isolated from a sewage treatment plant. J Environ Sci. 2008;20(12):1508–13.
[25] Monod J. The Growth of Bacterial Cultures. Annu Rev Microbiol. 1949;3(1):371–94.
[26] Boon B, Laudelout H. Kinetics of nitrite oxidation by Nitrobacter winogradskyi. Biochem J. 1962;85:440–7.
[27] Teissier, G. Growth of bacterial populations and the available substrate concentration. Rev Sci Instrum. 1942;3208:209–14.
[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] Han K, Levenspiel O. Extended Monod kinetics for substrate, product, and cell inhibition. Biotechnol Bioeng. 1988;32(4):430–7.
[31] Luong, J.H.T. Generalization of monod kinetics for analysis of growth data with substrate inhibition. Biotechnol Bioeng. 1987;29:242–8.
[32] Dawson RMC, Elliott DC, Elliott WH, Jones, K.M. Data for biochemical research. Oxford University Press, London; 1969.
[33] 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.
[34] López S, Prieto M, Dijkstra J, Dhanoa MS, France J. Statistical evaluation of mathematical models for microbial growth. Int J Food Microbiol. 2004;96(3):289–300.
[35] Akaike H. STATISTICAL INFORMATION PROCESSING SYSTEM FOR PREDICTION AND CONTROL. AAPG Bull Am Assoc Pet Geol. 1981;237–41.
[36] 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.
[37] Ross T, McMeekin TA. Predictive microbiology. Int J Food Microbiol. 1994;23(3-4):241–64.
[38] Bakhshi Z, Najafpour G, Kariminezhad E, Pishgar R, Mousavi N, Taghizade T. Growth kinetic models for phenol biodegradation in a batch culture of Pseudomonas putida. Environ Technol. 2011;32(16):1835–41.
[39] Sahinkaya E, Dilek FB. Modeling chlorophenols degradation in sequencing batch reactors with instantaneous feed-effect of 2,4-DCP presence on 4-CP degradation kinetics. Biodegradation. 2007;18(4):427–37.
[40] Wayman M, Tseng MC. Inhibition threshold substrate concentrations. Biotechnol Bioeng. 1976;18(3):383–7.
[41] Mulchandani A, Luong JHT, Groom C. Substrate inhibition kinetics for microbial growth and synthesis of poly-β-hydroxybutyric acid by Alcaligenes eutrophus ATCC 17697. Appl Microbiol Biotechnol. 1989;30(1):11–7.
[42] Głuszcz 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.
[43] Haldane JBS. Enzymes,. Longmans, Green and Co. London; 1930.