HIBISCUS PUBLISHER

JOURNAL OF BIOCHEMISTRY, MICROBIOLOGY AND BIOTECHNOLOGY

Website: https://journal.hibiscuspublisher.com/index.php/JOBIMB

JOBIMB VOL 8 NO 1 2020
SARS-Cov-2 protease

Utilization of Comprehensive Mathematical Modelling to Evaluate the Growth Kinetics of *Pseudomonas putida* LY1 on Phenol

Abubakar Aisami[1]* and Mohammed Maikudi Usman[2]

[1]Department of Biochemistry, Faculty of Sciences, Gombe State University, P. M. B. 027, Gombe, Nigeria.
[2]Department of Biotechnology, School of Life Science, Modibbo Adama University of Technology, Yola, Nigeria.

*Corresponding author:
Dr. Abubakar Aisami,
Department of Biochemistry,
Faculty of Sciences, Gombe State University,
P. M. B. 027, Gombe, Nigeria
Email: abubakar.aisami05@gmail.com

HISTORY

Received: 25th Feb 2020
Received in revised form: 14th of March 2020
Accepted: 18th of May 2020

KEYWORDS

Degradation kinetics
phenol
Pseudomonas
Haldane
AICc

ABSTRACT

Kinetic equations, which explain the behaviour of a microbe or an enzyme towards a specific substrate, are key to understanding many phenomena in biotechnological processes. They facilitate the mathematical prediction of growth parameters essential for the identification of key growth control parameters. We remodelled Banerjee and Ghoshal's published research (Banerjee and Ghoshal 2010) using some more kinetic growth models, such as Monod, Teissier, Andrews and Noack, Hinshelwood, Moser, Aiba, Webb (Edward), Yano and Koga, Han and Levenspiel and Luong used statistical methods such as Root Mean Square (RMSE), Adjusted Coefficient of Determination (R^2), corrected Akaike Information Criterion (AICc), Bias Factor, Accuracy Factor to determine the accuracy of the fitted model. The best model was Haldane with the true value of μ_{max} determined as the value where the gradient for the slope is zero was 0.115 h^{-1} at 51 mg/L phenol. The results indicate that the exhaustive use of mathematical models on available published results could gleam new optimal models that can provide new knowledge on the way toxic substance inhibits growth rate in microbes.

INTRODUCTION

Phenols and phenolic compounds are harmful to humans even at small concentrations, all of which are listed as hazardous substances for different reasons owing to their toxicity to human health [1,2]. Several of the phenolics includes chlorophénols, nitrophenols, methyl phenols, alkylphenols and aminophenols. Phenol is inhibitory to bacterial growth, which is expressed in decreased growth levels as the concentration of phenols increases [3]. The design and optimisation of biological transformation processes include quantitative empirical data. A selection of mathematical models has been developed to characterize the metabolism dynamics of compounds introduced to the pure culture of microorganism or natural environment microbial populations. A useful resource in biotechnology is the relationship between the specific growth rate (μ) of a microbial community and the substrate concentration (S). The Monod equation was commonly used to describe the rate of utilization of substrates linked to growth [4,5]. However, the original Monod model could not be used when a substratum shows inhibition against its biodegradation. In this case, instead, its derivatives have been invented that have new constants that provided substrate corrections. **Table 1** shows a variety of microbial growth of the kinetic model available for this work biodegradation. There are numerous kinds of literature which generalize the use of the Haldane model in the literature to model substratum inhibition to growth or degradation rate. This is even though many other models are more reliable for a single substrate-inhibiting compound such as phenol. Aside from the commonly documented Haldane model, for example [6]. Many other models, such as Luong [7–9] and Edward [10] were found to be optimal. In some circumstances, therefore, the use of extensive models available could replace the Haldane. Without directly applying these other models to the available data on growth or degradation rate and proper statistical evaluation, the Haldane model cannot be used exclusively in a liberal fashion.

The purpose of this work is therefore to analyze similarities and differences between models using published available data for additional detailed modelling and, based on statistical reasoning, to answer the question of which model(s) should be used. It would offer fresh evidence and results that could spurn and expose new knowledge and changes in researchers' work already completed.

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

Author	Degradation Rate	Author
Monod	$q_{\max}\frac{S}{K_s+S}$	[11]
Haldane	$q_{\max}\frac{S}{S+K_s+\frac{S^2}{K_i}}$	[12]
Teissier	$q_{\max}\left(1-\exp\left(-\frac{S}{K_i}\right)-\exp\left(\frac{S}{K_s}\right)\right)$	[13]
Aiba	$q_{\max}\frac{S}{K_s+S}\exp(-KP)$	[14]
Yano and Koga	$\frac{q_{\max}S}{S+K_s+\left(\frac{S^2}{K_1}\right)\left(1+\frac{S}{K}\right)}$	[15]
Han and Levenspiel	$q_{\max}\left[1-\left(\frac{S}{S_m}\right)\right]^n\left[\frac{S}{S+K_s\left(1-\frac{S}{S_m}\right)^m}\right]$	[16]
Luong	$q_{\max}\frac{S}{S+K_s}\left[1-\left(\frac{S}{S_m}\right)^n\right]$	[17]

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

MATERIALS AND METHODS

Acquisition of Data

The graph showing the degradation rate against substrate phenol concentration [18] for *Pseudomonas putida* LY1 in Figure 4 was processed electronically using WebPlotDigitizer 2.5 [19], which helps digitize scanned plots into the data table with sufficient accuracy [20].

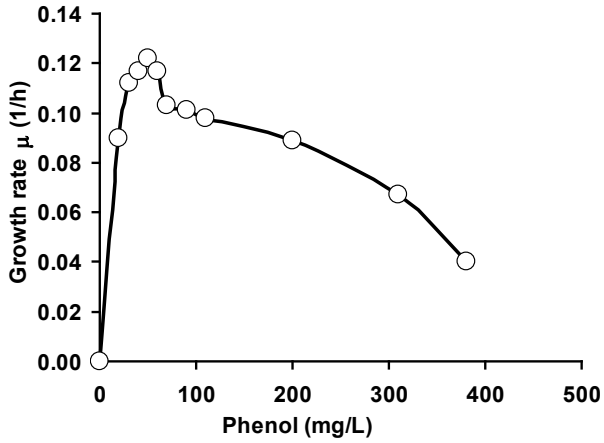

Fig 1. Replotted data of the degradation rate against substrate phenol concentration for *Pseudomonas putida* LY1.

Fitting of the data

Via nonlinear regression, the nonlinear equations were fitted to growth data with a Marquardt algorithm that minimizes residual square sums using CurveExpert Professional software (version 1.6). It is a search method to minimize the sum of the differences between the expected and observed values in the squares. The software determines starting values automatically by looking for the steepest elevation of the curve between four datum points (estimation of μ_{max}), intersecting this line with the x-axis (prediction of half) and taking the final data point as an asymptote (A) estimate. The model of the Huang needs to be numerically solved, as it is a differential equation. The differential equation was numerically resolved using the Runge-Kutta method. To solve this equation a differential equation solver (ode45) was used in MATLAB (version 7.10.0499, The MathWorks, Inc., and Natick, MA).

Statistical analysis

To assess whether there is a statistically significant difference between models with different parameters, the consistency of fit to the same experimental data was statistically tested using various methods such as the root-mean-square error (RMSE), the adjusted determination coefficient (R2), the bias factor (BF), the accuracy factor (AF), (R^2), bias factor (BF), accuracy factor (AF), corrected AICc (Akaike Information Criterion) and F-test [21].

The RMSE has been calculated according to the Eq. (1) where Pdi is the values predicted by the model and Obi are experimental data, n is the number of experimental data and p is the number of parameters of the model is evaluated. The smaller number of parameters of the model is expected to give smaller RMSE values.

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

The coefficient of determination or R^2 is used in linear regression models to assess the quality of fit of the model. However, in the case of nonlinear regression, where the difference in the number of parameters between one model and another is normal, the adoption of the method does not readily provide comparative analysis. The adjusted R^2 is therefore used to calculate the quality of nonlinear models according to the formula where the RMS is the Residual Mean Square and S_y^2 is the total variance of the y-variable.

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

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

The Akaike Information Criterion (AIC) offers a system of model selection by evaluating the quality of a given statistical model for a given set of experimental data [22]. AIC deals with the trade-off concerning the fitness of the model as well as the complexity of the model. It is based on the theory of information. The method provides a relative approximation of the information lost for each time a model is used to represent a process that generates information or data. The most preferred model would be the model showing the minimum value for AIC for the output of a set of predicted models. This value is often a negative value,

with, for example, an AICc value of-10 more preferred than that of-1. The equation includes the number of penalty parameters, the more parameters, the lower the output preference or the higher the AIC value. The most preferred model would be the model showing the minimum value for AIC for the output of a set of predicted models. This value is often a negative value, with, for example, an AICc value of-10 more preferred than that of-1. The equation includes the number of penalty parameters, the more parameters, the lower the output preference or the higher the AIC value.

AIC therefore not only rewards fitness but also does not encourage the use of more complicated models (overfitting) for fitting experimental data. Since the data in this work is small compared to the number of parameters used in the 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;

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

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

The analysis takes into consideration the change in fitness and the difference in the number of parameters between the two models. The model with the smallest AICc value is most probably correct for each data set [23].

Accuracy Factor (AF) and Bias Factor (BF) to test for the goodness-of-fit of the models as suggested by Ross [25] were also used. The identical to1 Bias factor indicates a perfect match between the predicted and observed values. A bias factor with values < 1 indicates a fault-dangerous model for microbial growth curves or degradation studies while a bias factor with values > 1 indicates a fail-safe model. The accuracy factor is usually as high as 1 and higher AF values are less accurate predictions.

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

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

RESULTS AND DISCUSSION

The results of the curve fitting are shown in Figures 2 to 6. The model from Han and Levenspiel did not fit the experimental data and was excluded. All other models tested except the Monod model offered a relatively good fit based on visual observation.

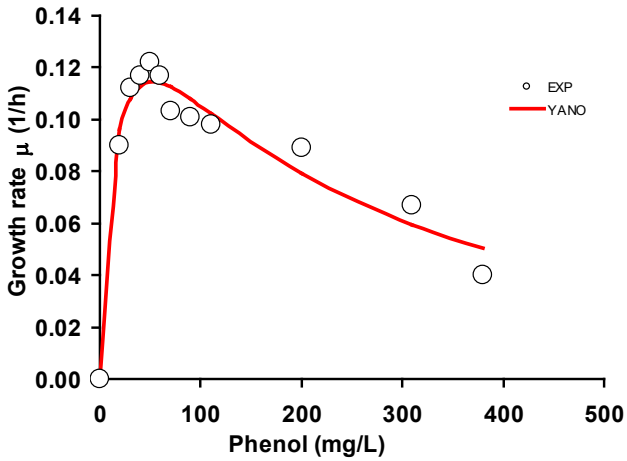

Fig. 2. Fitting experimental data with the Yano model.

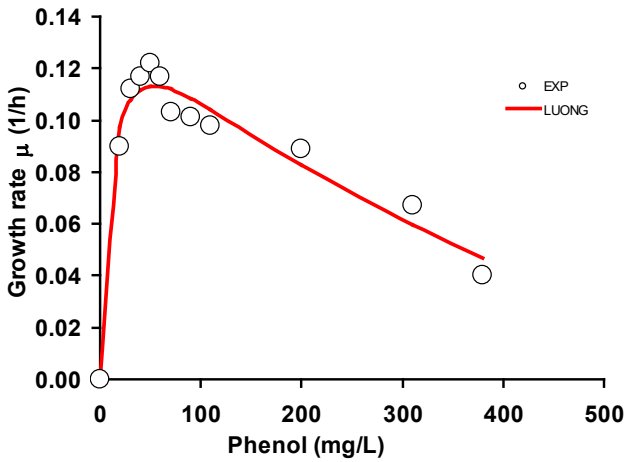

Fig. 3. Fitting experimental data with the Luong model.

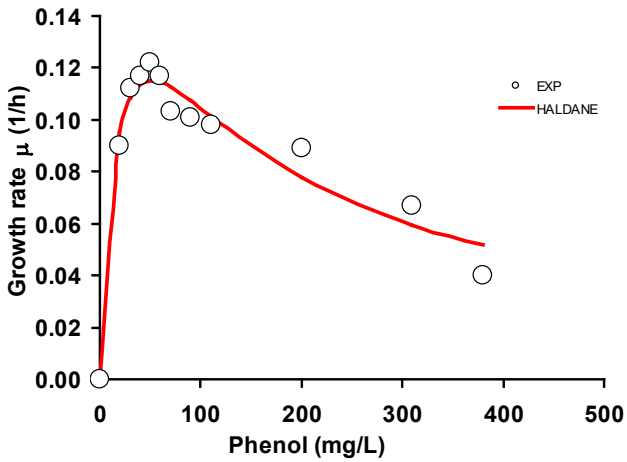

Fig. 4. Fitting experimental data with the Haldane model.

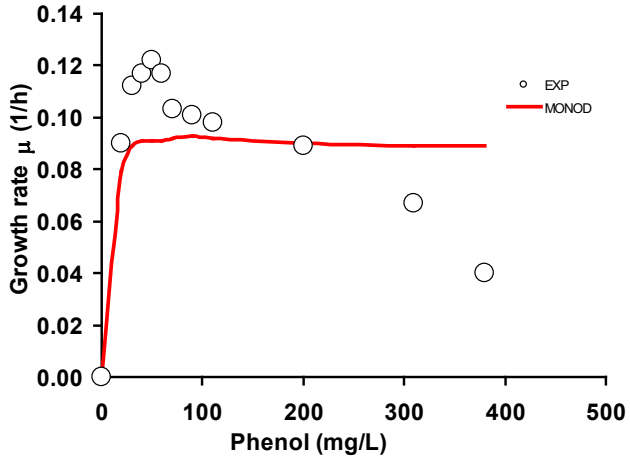

Fig. 5. Fitting experimental data with the Monod model.

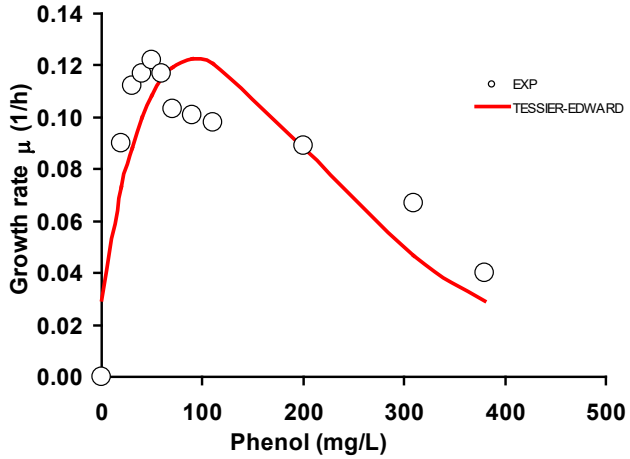

Fig. 6. Fitting experimental data with the Teissier-Edward model**.**

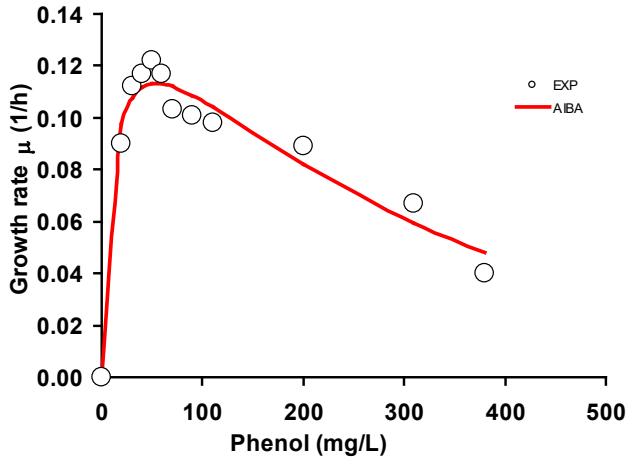

Fig. 7. Fitting experimental data with the Aiba model.

The statistical and accuracy analysis of all the six kinetic models used, Haldane was showed to the best model dues to its lower RMSE and AICc values, highest adjusted R^2 values, F-test and with Bias Factor and Accuracy Factor nearest to unity (1.0) (**Table 2**). The Haldane model was also stated by Li et al. [18], although in the original work, only Haldane was evaluated. The calculated value for the Haldane constants; i.e. maximal growth rate, half-saturation constant for maximal growth and inhibition constant defined as μ_{max}, K_s and K_i were similar to those found by Li et al. [18]. It should be noted that the value of μ_{max} obtained based on curve fitting linearization is not the true μ_{max} value as the true value should be where the gradient for the slope is zero and in this case, the value was approximately 0.115 h^{-1} at 51 mg/L phenol, which is new information not carried out in the original work.

Table 2. Statistical analysis of kinetic models.

Model	*p*	RMSE	R2	adR2	AICc	BF	AF
Luong	4	0.0079	0.963	0.943	-93	1.005	1.072
Yano	4	0.0084	0.959	0.936	-92	1.008	1.078
Tessier-Edward	3	0.0211	0.700	0.587	-76	0.928	1.180
Aiba	3	0.0260	0.232	-0.056	-71	0.974	1.233
Haldane	3	0.0079	0.959	0.943	-100	1.008	1.077
Monod	2	0.0247	0.190	0.010	-78	0.974	1.233
Han and Levenspiel	5	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

Note:
p No of parameter
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

Table 3. Inhibition kinetics parameters from the Haldane model

	μ_{max} (h^{-1})	K_s (mg/L)	K_i (mg/L)
Value	0.2000	20.0000	129.83
Std Err	0.0400	9.5200	45.82
95% confidence interval	0.11 to 0.29	-1.54 to 41.54	26.18 to 233.48

Most studies on substrate inhibition of microbial growth are performed using toxic substrates such as aromatic and halogenated hydrocarbons [26,27] and therefore it can be deduced that the growth rate at high concentrations will be heavily affected and the ordinary use of the Monod model will fail. There were other models for describing substrate inhibition kinetics developed during this period such as the discontinuous models of Wayman and Tseng [28]. The reason for the development of the discontinuous model is the previous models developed such as Haldane, Andrews and Noack, and Webb can describe the 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 [29]. A continuous version of the above models developed by Luong has found popular support due to its close agreement to experimental data in many cases [7,8,30] including this one. The key feature of the Luong model is its ability to predict accurately the S_m value of the maximum concentration of substrates above which growth is completely inhibited.

CONCLUSION

Both growth and degradation 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 Haldane model is the best model in fitting the degradation kinetics data from *Pseudomonas putida* LY1. We predicted that many existing published models in the literature could be better modelled using the various kind of growth or degradation models available instead of the ubiquitous Haldane model for instance.

REFERENCES

1. Christen P, Vega A, Casalot L, Simon G, Auria R. Kinetics of aerobic phenol biodegradation by the acidophilic and hyperthermophilic archaeon *Sulfolobus solfataricus* 98/2. Biochem Eng J. 2012;62:56–61.
2. Hasan SA, Jabeen S. Degradation kinetics and pathway of phenol by *Pseudomonas* and *Bacillus* species. Biotechnol Biotechnol Equip. 2015;29(1):45–53.
3. Aisamia A, Yasida NA, Joharic WLW, Ahmada SA, Shukora MY. Growth rate abolishment on phenol as a substrate by *Pseudomonas* sp. AQ5-04 best modelled using the Luong substrate inhibition kinetics. DESALINATION WATER Treat. 2019; 152:214–220.
4. Kiviharju K, Salonen K, Leisola M, Eerik T. Modeling and simulation of *Streptomyces peucetius var. caesius* N47 cultivation and ε-rhodomycinone production with kinetic equations and neural networks. J Biotechnol. 2006;126:365–373.
5. Shokrollahzadeh S, Bonakdarpour ÆB. Growth kinetics and Pho84 phosphate transporter activity of *Saccharomyces cerevisiae* under phosphate-limited conditions. J Ind Microbiol Biotechnol. 2007;34:17–25.
6. 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 Jan;53(1):9–19.
7. 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.
8. 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.
9. Aisami A, Yasid NA, Johari WLW, Shukor MY. Estimation of the Q10 value; the temperature coefficient for the growth of Pseudomonas sp. aq5-04 on phenol. Bioremediation Sci Technol Res. 2017;5(1):24–26.
10. Saravanan P, Pakshirajan K, Saha P. Batch growth kinetics of an indigenous mixed microbial culture utilizing m -cresol as the sole carbon source. J Hazard Mater. 2009;162:476–481.
11. Monod J. The Growth of Bacterial Cultures. Annu Rev Microbiol. 1949;3(1):371–94.
12. Boon B, Laudelout H. Kinetics of nitrite oxidation by Nitrobacter winogradskyi. Biochem J. 1962;85:440–7.
13. Teissier G. Growth of bacterial populations and the available substrate concentration. Rev Sci Instrum. 1942;3208:209–14.
14. Aiba S, Shoda M, Nagatani M. Kinetics of product inhibition in alcohol fermentation. Biotechnol Bioeng. 1968 Nov 1;10(6):845–64.
15. Yano T, Koga S. Dynamic behavior of the chemostat subject to substrate inhibition. Biotechnol Bioeng. 1969 Mar 1;11(2):139–53.
16. Han K, Levenspiel O. Extended Monod kinetics for substrate, product, and cell inhibition. Biotechnol Bioeng. 1988;32(4):430–7.
17. Luong JHT. Generalization of monod kinetics for analysis of growth data with substrate inhibition. Biotechnol Bioeng. 1987;29(2):242–8.
18. Li Y, Li J, Wang C, Wang P. Growth kinetics and phenol biodegradation of psychrotrophic *Pseudomonas putida* LY1. Bioresour Technol. 2010 Sep 1;101(17):6740–4.
19. Rohatgi A. Web Plot Digitizer. ht tp. Arohatgi InfoWebPlotDigitizeraccessed Aug 2014. 2014;154.
20. De Stefano LA, Stepanov II, Abramson CI. The first order transfer function in the analysis of agrochemical data in honey bees (Apis mellifera L.): Proboscis extension reflex (PER) studies. Insects. 2014;5(1):167–198.
21. 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.
22. Akaike H. Factor analysis and AIC. Psychometrika. 1987;52:371–386.
23. Motulsky HJ, Ransnas LA. Fitting curves to data using nonlinear regression: a practical and nonmathematical review. FASEB J. 1987;1(5):365–374.
24. Motulsky HJ, Ransnas LA. Fitting curves to data using nonlinear regression: a practical and nonmathematical review. Fed Am Soc Exp Biol. 1987; 1(5):365–374.
25. Ross T, McMeekin TA. Predictive microbiology. Int J Food Microbiol. 1994; 23(3–4):241–64.
26. 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*. 2011;3330(November 2016).
27. Sahinkaya E, Dilek ÆFB. Modeling chlorophenols degradation in sequencing batch reactors with instantaneous feed-effect of 2 , 4-DCP presence on 4-CP degradation kinetics. Biodegradation. 2007;427–437.
28. Wayman M, Tseng MC. Inhibition threshold substrate concentrations. Biotechnol Bioeng. 1976;18(3):383–7.
29. 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.
30. 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.