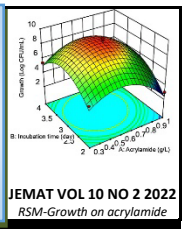
JEMAT 2022, Vol 10, No 2, 63-67
https://doi.org/10.54987/jemat.v10i2.789

HIBISCUS PUBLISHER

JOURNAL OF ENVIRONMENTAL MICROBIOLOGY AND TOXICOLOGY

Website: http://journal.hibiscuspublisher.com/index.php/JEMAT/index



Mathematical Modeling of the Inhibition Kinetics of Malachite Green Decolorization by *Staphylococcus aureus*

Motharasan Manogaran[1], Wan Yudreina Yudryk Wan Azni[1], Nur Muhamad Syahir Abdul Habib[1] and Nur Adeela Yasid[1]*

[1]Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

*Corresponding author:
Nur Adeela Yasid
Department of Biochemistry,
Faculty of Biotechnology and Biomolecular Sciences,
Universiti Putra Malaysia,
43400 UPM Serdang, Selangor, Malaysia.
Tel: 603-89466722
Email: adeela@upm.edu.my

HISTORY

Received: 15th Oct 2022
Received in revised form: 12th Dec 2022
Accepted: 27th Dec 2022

KEYWORDS

Malachite Green
Triphenylmethane dye
Decolourization
Staphylococcus aureus
Hans-Levenspiel

ABSTRACT

Basic Green 4 or Malachite Green (MG) is an important dye that found great usage in controlling fish pathogens. The use of MG has been banned but developing, and third-world countries still found applications for this dye. Bioremediation of dyes using microorganisms is on the rise. The ability to accurately predict the rate of bioremediation relies upon the gathering of the accurate rate of decolourisation, which is often inhibited at high concentrations of the toxicant. Various secondary models such as Monod, Haldane, Teissier, Aiba, Yano and Koga, Hans-Levenspiel, Webb and the Luong models were utilized to fit the specific decolourisation rate, and most of them show visually acceptable fitting except Monod and Teissier. The best model based on statistical analysis was Hans-Levenspiel with the highest value for the adjusted coefficient of determination and the lowest values for RMSE, AICc, HQC and BIC and the closest value to 1.0 for accuracy and bias factors. The Hans-Levenspiel model was found to conform to normality tests and is adequate to be used to fit the experimental data. The normality tests carried out using tests such as the Kolmogorov-Smirnov, Wilks-Shapiro and the D'Agostino-Pearson omnibus K2 test shows that the model pass the normality tests with $p > 0.05$ for all normality tests carried out. The experimental data obtained indicates that Malachite Green is toxic and slows down the rate of decolourisation at higher concentrations. The maximum MG specific biodegradation rate (q_{max}), half-saturation concentration (K_S), maximum allowable MG concentration (S_m), and the shape factors (n and m) were 0.136 h^{-1}, 0.56 mg/L, 2691 mg/L, -33.31 and 35.12, respectively. The parameters obtained from this exercise can be utilized to model the bioremediation of MG in the future.

INTRODUCTION

Even though dyes perform a considerable function inside the manufacturing and industrial areas, pollution by dye will take place as a result of incorrect waste management. Despite the fact that natural dyes have got no recourse in any way, nonetheless, these synthetic dyes might cause toxicity to both humans and animals. As a consequence of fast industrialization, dyes are getting into the main water supply affecting the environment. These neglected textile effluents are packed with colour, high in BOD or biochemical oxygen demand, high in COD or chemical oxygen demand, high in TOC or total organic carbon, elevated SS or suspended solids, temperature, pH, turbidity and toxicity [1].

Bioremediation can solve the problem of contaminants of soil, water or sediments, and it is the productive utilisation of the biodegradative method to eliminate or detoxify pollutants that go into the environment and jeopardize the public health or security of the surroundings [2,3]. Within this framework, bioremediation is the utilization of organisms to break down, sequester or conjugate environmental contaminants.

Particular microorganisms are capable of breaking down toxins in the surroundings, which has been well known in the dyes field. In the last decades, the capacity of microorganisms has been looked into as an approach to degrade, decolourise, convert and mineralise dyes to safe, non-toxic by-products. In addition, the function of microorganisms employed for dye degradation is eco-friendly as a much less chemical substance is utilized to get rid of the contaminated site. In addition to this,

whenever less chemical is included, lower energy is needed for the bioremediation process, thus rendering it a cost-effective substitute for both chemical and physical decomposition procedures. The inhibitory effect of dye or its degradation metabolite to the growth or degradation rate of the dye can be modelled using secondary models such as Haldane, which is popular due to its simple equation and has been reported in several studies [4–6] despite the existence of numerous other secondary models such as Teissier, Aiba, Yano and Koga, Hans-Levenspiel, Webb and the Luong that can predict concentrations of the toxicant that can completely cease growth or degradation rate.

In a previous work, a recalcitrant dye; Basic Green 4 or malachite green (MG) is degraded by *Staphylococcus* aureus and hence has the potential to be a remediation agent. The rate of decolourisation appears to be inhibited by a high concentration of the dye. Hence, the objective of this research is to mathematically model the degradation or the decolourization of Malachite Green dye using non-linear regression such as Monod, Haldane, Teissier, Aiba, Yano and Koga, Hans-Levenspiel, Webb and the Luong models. This modelling will allow for more accurate parameters of decolourization to be obtained. The best model will be evaluated based on the various statistical test such as the adjusted coefficient of determination ($adjR^2$), root means square error (RMSE), corrected Akaike Information Criterion (AICc), accuracy factor (AF) and bias factor (BF).

MATERIALS AND METHODS

Data acquisition

Graphical data of a published work [7] from Figure 7 were electronically processed using WebPlotDigitizer 2.5 [8] which helps to digitize scanned plots into a table of data with good precision and reliability [9,10].

Fitting of the data

The data were fitted using a nonlinear regression that uses a Marquardt algorithm (**Table 1**). CurveExpert Professional software (Version 1.6), which minimizes the sums of the square of the differences between values of the predicted and measured.

Statistical analysis

The root-mean-square error or RMSE is the standard deviation of the residuals (prediction errors), and it measures the spread of the residual. It is calculated according to **Eq. 1**, where p is the number of parameters of the assessed model, Ob_i is the experimental data, Pd_i is the values predicted by the model and n is the number of experimental data.

The RMSE was calculated as folows,

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

where

n number of experimental data
Pd_i pvioleticted values by the model
Ob_i experimental data
p parameters number of the model

Table 1. Kinetic models for the growth of *Staphylococcus aureus* on dyes.

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]
Edward (Webb)	$q_{\max}\frac{S\left(1+\frac{S}{K}\right)}{S+K_s+\left(\frac{S^2}{Ki}\right)}$	[17]
Luong	$q_{\max}\frac{S}{S+K_s}\left[1-\left(\frac{S}{S_m}\right)^n\right]$	[18]

Note:
q_{max} maximal decolourisation 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)

This error function penalizes for a number of parameters, and as a rule of thumb, the model with the smaller number of parameter resulted in a smaller RMSE value [19] and is more desired than a larger RMSE value. In linear and nonlinear regression, the assessment of the goodness of fit is often based on the coefficient of determination or R^2. However, the method ignores the number of parameters of models and hence, does not freely provide comparative analysis. As a solution, and adjusted R^2 that takes into account the number of parameter of models (**Eqns. 2** and **3)** is used to work out the quality of nonlinear models according to the formula below;

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

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

where

S_y^2 is the total variance of the y-variable and RMS is the Residual Mean Square

The Akaike information criterion (AIC) is an estimator of the relative quality of statistical models. It is established upon information theory. The error function trade-off goodness of fit of models taking into account the number of parameter of the model. To select for the best model, the model with the least value for AIC is the best. When the data in a study is small concerning the parameters' number, a corrected version of AIC; the Akaike information requirements (AIC) with the correction or AICc is utilised instead [20].

Only the quantum of the difference is important and not the actual values with a difference of 5 usually indicates a more likelihood of the data with the smaller value to be accurate or correct. The formula incorporates some variables penalty where the more the variables, the higher the AIC value indicating a less parsimonious model. AIC discourage the use of more complicated models (overfitting) in fitting experimental data. AICc is calculated using the following equation (**Eqn. 4**);

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

Where
n number of data points
p parameter numbers of the model

Another goodness-of-fit of models is the Accuracy Factor (AF), and Bias Factor (BF) (**Eqns. 5** and **6**) adapted from common use in predicted microbiology for bacterial growth in food science [21]. The statistics calculates the perfect match between experimental and predicted values. As a rule, a BF value > 1.0 indicates a model which is fail-safe a value < 1.0 indicates a model that is fail-dangerous. On the other hand, the AF is always ≥ 1.0, with precise models giving values nearing to 1.0.

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

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

Assesment of normality (**Eqn. 7**) for the residuals was carried out using the GraphPad Prism® 6 (Version 6.0, GraphPad Software, Inc., USA). The residual for the i^{th} observation in the regression model can be mathematically represented as follows;

$$e_i = y_i - f(x_i;\hat{\beta}) \quad \textbf{(Eqn. 7)}$$

Where the i^{th} response from a given data set is denoted by y_i while at each set of the i^{th} observation, the vector for the explanatory variables is x_i [22], the normality tests carried out is based on the tests of Kolmogorov-Smirnov [23,24], Wilks-Shapiro [25] and the D'Agostino-Pearson omnibus K2 test [26].

RESULT AND DISCUSSION

Various secondary models (**Figs. 1 to 8**) were utilized to fit the specific decolourisation rate, and most of them show visually acceptable fitting except Monod and Teissier.

The best model based on statistical analysis was Hans-Levenspiel with the highest value for the adjusted coefficient of determination and the lowest values for RMSE, AICc, HQC and BIC and the closest value to 1.0 for accuracy and bias factors. The Hans-Levenspiel model was found to conform to normality tests and is adequate to be used to fit the experimental data. The normality tests carried out show that the model passes the normality tests with p >0.05 for all normality tests carried out [22]. The experimental data obtained indicates that Malachite Green is toxic and slows down the rate of decolourisation at higher concentrations. The maximum MG specific biodegradation rate (q_{max}), half-saturation concentration (K_S), maximum allowable MG concentration (S_m), and the shape factors (n and m) were 0.136 h^{-1}, 0.56 mg/L, 2691 mg/L, -33.31 and 35.12, respectively.

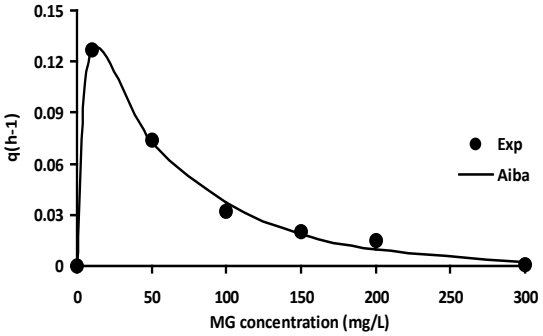


Fig. 1. Fitting the effect of Malachite Green dye concentration on the specific growth rate of *Staphylococcus aureus* using the Aiba model.

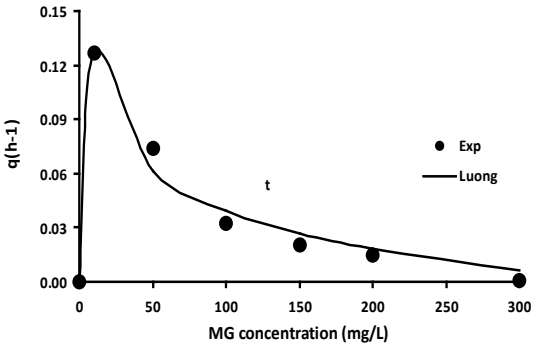


Fig. 2. Fitting the effect of Malachite Green dye concentration on the specific growth rate of *Staphylococcus aureus* using the Luong model.

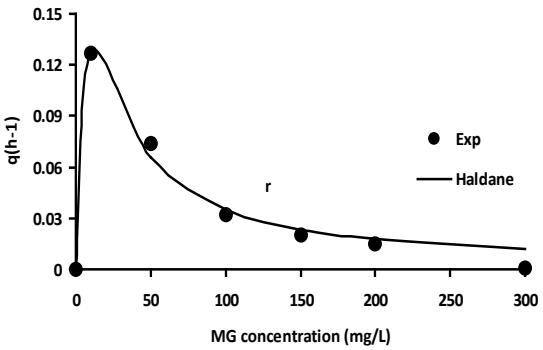


Fig. 3. Fitting the effect of Malachite Green dye concentration on the specific growth rate of *Staphylococcus aureus* using the Haldane model.

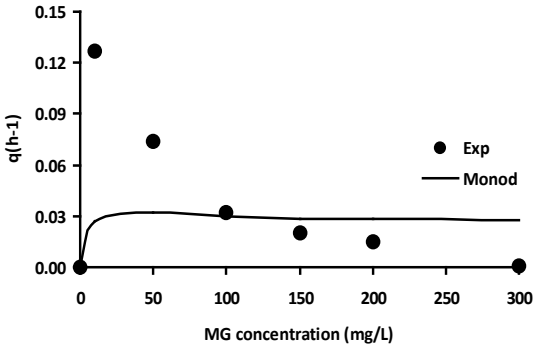


Fig. 4. Fitting the effect of Malachite Green dye concentration on the specific growth rate of *Staphylococcus aureus* using the Monod model.

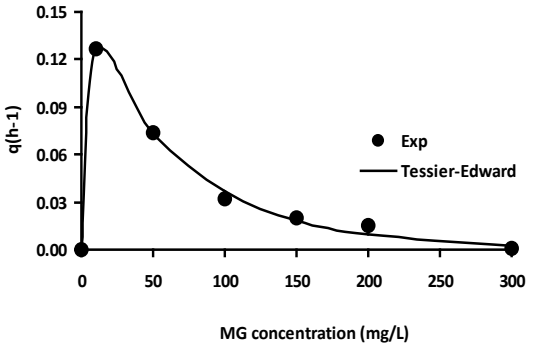


Fig. 5. Fitting the effect of Malachite Green dye concentration on the specific growth rate of *Staphylococcus aureus* using the Tessier-Edward model.

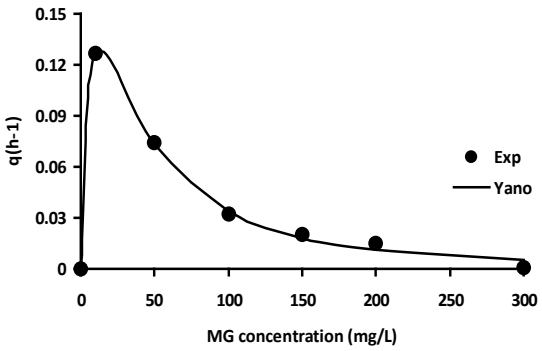


Fig. 6. Fitting the effect of Malachite Green dye concentration on the specific growth rate of *Staphylococcus aureus* using the Yano model.

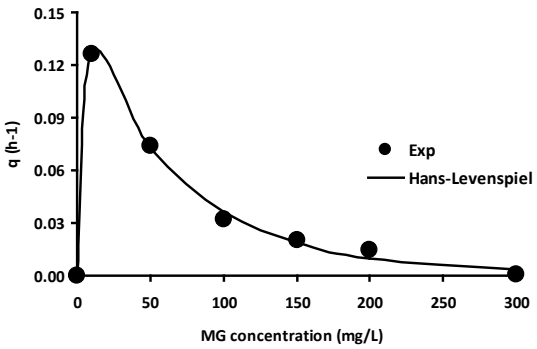


Fig. 7. Fitting the effect of Malachite Green dye concentration on the specific growth rate of *Staphylococcus aureus* using the Hans-Levenspiel model.

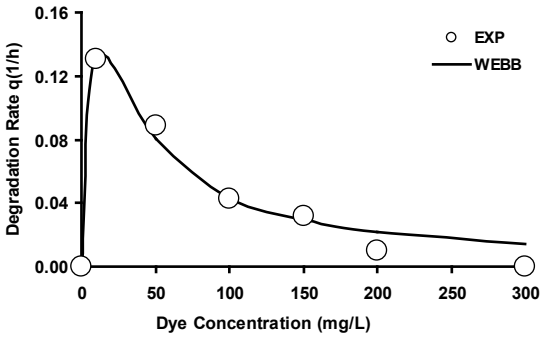


Fig. 8. Fitting the effect of Malachite Green dye concentration on the specific growth rate of *Staphylococcus aureus* using the Webb model.

Table 2. Statistical analysis of kinetic models.

model	p	RMSE	AdjR^2	AICc	BF	HQC	BIC	AF
Aiba	3	0.003	0.9944	-85.74	1.94	-92.68	-92.06	2.068
Luong	4	0.0066	0.9697	-73.07	1.78	-80.01	-79.4	1.865
Haldane	3	0.0065	0.9679	-73.12	5.59	-80.06	-79.44	3.386
Monod	2	0.0223	0.5765	-53.53	6.36	-60.47	-59.85	4.783
Tessier-Edward	3	0.0318	0.4008	-47.85	0	-54.79	-54.17	nil
Yano	4	0.0019	0.9977	-92.89	1.77	-99.83	-99.21	1.36
Hans-Levenspiel	5	0.008	0.9996	-106.2	1.1	-113.1	-112.5	1.1
Webb	4	0.0063	0.97	-73.66	2.88	-80.59	-79.98	1.888

Note:
p parameter
RMSE Root Mean Square Error
R^2 Coefficient of Determination
adR^2 Adjusted Coefficient of Determination
AICC Corrected Akaike Information Criterion
BF Bias Factor
AF Accuracy Factor

Mathematical modelling on the effect of substrate (dyes) on the growth rate of dye-degrading bacteria is rarely done despite this kind of exercise being routinely carried out in other xenobiotics-degrading microorganism works. The model parameters obtained from such exercise can be a useful tool in comparing efficiency between degraders and can be used to estimate the inhibitory effect of the substrate in field studies. The Hans-Levenspiel model was also the best model for the biodegradation of methylene blue (MB) by *Ralstonia eutropha*. The maximum MB specific biodegradation rate (r_{max}), half-saturation concentration of MB (Ks), maximum allowable MB concentration (S_m), and the shape factors (n and m) were 7.37 mg h^{-1}, 32.13 mg/L, 158.8 mg/L, 0.27, and 0.76, respectively [27].

The Haldane model is often the one that is used as the basis for most dye-decolorizing kinetics research. For instance, in the process of Congo red being broken down by Bacillus species, the Haldane and Monod models were applied, and it was discovered that the Haldane model was the superior of the two [28]. The Haldane model was also utilized as the best model for the bio-decolorization of the textile azo dye Reactive Red 2 by a mixed, mesophilic methanogenic culture [4] and the bio-degradation of Tectilon Yellow 2G (TY2G) by a *Pseudomonas putida* mutant [29].

In the biodegradation of Methyl Orange (MO) with tolerance at concentrations of up to 100 mg/L by the salt-tolerant *Bacillus* sp. strain CICC 23870 the biodegradation was estimated by the Haldane model as the sole model due to the popularity of this model. The average specific decolorization rate of the free cell system was 26.30 mg/g/h at an initial MO concentration of 32.7 mg/L [30].

Because the Haldane model has been proved to be more accurate when compared to other models, this generalization regarding the employment of the Haldane model in published works ought to be approached with extreme caution. For example, in addition to the Haldane model, which is most frequently stated [31], several other different models have been found to be optimal such as Luong [32,33] and Edward [34]. As a consequence of this, the utilization of extensive models that are easily available may therefore replace the Haldane in certain circumstances. The unique utilization of the Haldane model must not be used freely because it can only be achieved by really fitting these other models to the data that is available for either the growth or degradation rate, and then conducting the proper statistical analysis.

CONCLUSION

Malachite Green (MG) is an important dye in controlling fish pathogens as it is antibacterial, antifungal, and anti-parasitic. Bioremediation of MG using microorganisms is on the rise. The rate of decolourisation, which is often inhibited at high concentrations of toxicant can be modelled using various secondary models. The best model based on statistical analysis was Hans-Levenspiel with the highest value for the adjusted coefficient of determination and the lowest values for RMSE, AICc, HQC and BIC and the closest value to 1.0 for accuracy and bias factors. The Hans-Levenspiel model was found to conform to normality tests and is adequate to be used to fit the experimental data. The experimental data obtained indicates that Malachite Green is toxic and slows down the rate of decolourisation at higher concentrations. The maximum MG specific biodegradation rate (q_{max}), half-saturation concentration (K_S), maximum allowable MG concentration (S_m), and the shape factors (n and m) were 0.136 h^{-1}, 0.56 mg/L, 2691 mg/L, -33.31 and 35.12, respectively. The parameters obtained from this exercise can be utilized to model the bioremediation of MG in the future.

REFERENCE

1. Pang YL, Abdullah AZ. Current Status of Textile Industry Wastewater Management and Research Progress in Malaysia: A Review. CLEAN – Soil Air Water. 2013 Aug 1;41(8):751–64.
2. Thapa B, Kc AK, Ghimire A. A Review On Bioremediation Of Petroleum Hydrocarbon Contaminants In Soil. Kathmandu Univ J Sci Eng Technol. 2012;8(1):164–70.
3. Ang EL, Zhao H, Obbard JP. Recent advances in the bioremediation of persistent organic pollutants via biomolecular engineering. Enzyme Microb Technol. 2005 Oct 3;37(5):487–96.
4. Inan Beydilli M, Pavlostathis SG. Decolorization kinetics of the azo dye Reactive Red 2 under methanogenic conditions: effect of long-term culture acclimation. Biodegradation. 2005 Mar 1;16(2):135–46.
5. Srinivasan R, Kathiravan MN, Gopinath KP. Degradation of Tectilon Yellow 2G by hybrid technique: Combination of sonolysis and biodegradation using mutant Pseudomonas putida. Bioresour Technol. 2011;102(3):2242–7.
6. Uba G, Abubakar A, Ibrahim S. Optimization of Process Conditions for Effective Degradation of Azo Blue Dye by Streptomyces sp. DJP15: A Secondary Modelling Approac. Bull Environ Sci Sustain Manag. 2021 Dec 31;5(2):28–32.
7. Sandhiya R, Begum KS, Charumathi D. Decolourization of triphenylmethane dyes and dye industry effluent by *Staphylococcus aureus* isolated from dye contaminated site. Int J Pharm Pharm Sci. 2016 Sep 1;258–66.
8. Rohatgi A. WebPlotDigitizer—Extract Data from Plots, Images, and Maps [Internet]. 2018 [cited 2019 Jan 1]. Available from: http://arohatgi. info/WebPlotDigitizer
9. 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 Sep 29;2(1):1–5.
10. Manogaran M, Yasid NA, Ahmad SA. Mathematical modelling of glyphosate degradation rate by Bacillus subtilis. J Biochem Microbiol Biotechnol. 2017 Jul 31;5(1):21–5.
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. Webb JL. "Enzymes and Metabolic Inhibitors." In: Boston: Academic Press. 1963.
18. Luong JHT. Generalization of monod kinetics for analysis of growth data with substrate inhibition. Biotechnol Bioeng. 1987;29(2):242–8.
19. Halmi, MIE, ,Shukor MS, Johari W.L.W WLW, Shukor MY. Mathematical Modeling of the Growth Kinetics of *Bacillus* sp . on Tannery Effluent Containing Chromate. J Environ Bioremediation Toxicol. 2014;2(1):6–10.
20. Akaike H. Factor analysis and AIC. Psychometrika. 1987;52(3):317–32.
21. Ross T, McMeekin TA. Predictive microbiology. Int J Food Microbiol. 1994;23(3–4):241–64.
22. Halmi MIE, Shukor MS, Masdor NA, Shamaan NA, Shukor MY. Testing the normality of residuals on regression model for the growth of *Paracoccus* sp. SKG on acetonitrile. J Environ Bioremediation Toxicol. 2015;3(1):15–7.
23. Kolmogorov A. Sulla determinazione empirica di una legge di distribuzione. G Dell' Ist Ital Degli Attuari. 1933;4:83–91.
24. Smirnov N. Table for estimating the goodness of fit of empirical distributions. Ann Math Stat. 1948;19:279–81.
25. Royston P. Wilks-Shapiro algorithm. Appl Stat. 1995;44(4):R94.
26. D'Agostino RB. Tests for Normal Distribution. In: D'Agostino RB, Stephens MA, editors. Goodness-Of-Fit Techniques. Marcel Dekker; 1986.
27. Habibi A, Mehrabi Z. Aerobic degradation of methylene blue from colored effluents by *Ralstonia eutropha*. Pollution. 2017 Jul 1;3(3):363–75.
28. Gopinath KP, Asan Meera Sahib H, Muthukumar K, Velan M. Improved biodegradation of Congo red by using *Bacillu* sp. Bioresour Technol. 2009 Jan 1;100(2):670–5.
29. Srinivasan R, Kathiravan MN, Gopinath KP. Degradation of Tectilon Yellow 2G by hybrid technique: Combination of sonolysis and biodegradation using mutant *Pseudomonas putida*. Bioresour Technol. 2011 Feb 1;102(3):2242–7.
30. Yu L, Zhang X yu, Tang Q wen, Li J, Xie T, Liu C, et al. Decolorization characteristics of a newly isolated salt-tolerant Bacillus sp. strain and its application for azo dye-containing wastewater in immobilized form. Appl Microbiol Biotechnol. 2015 Nov 1;99(21):9277–87.
31. 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.
32. Hamitouche AE, 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.
33. 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.
34. 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(1):476–81.