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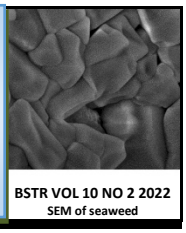

Mathematical Modeling of Substrate Inhibition Kinetics of *Staphylococcus aureus* Growth on Basic Violet 3

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ABSTRACT

Crystal violet or gentian violet or basic violet 3 (BV) is an essential dye utilized as a dye for textiles and paper, as well as being an ingredient in inks used for printing, ballpoint pens, and inkjet printers. In some cases, it is utilized for the purpose of imparting color to a variety of items, including antifreeze, fertilizer, detergent, and leather. The use of microorganisms for the purpose of BV bioremediation is becoming increasingly common. A number of secondary models, including Monod, Haldane, Teissier, Aiba, Yano and Koga, Hans-Levenspiel, Webb, and the Luong model, can be used to estimate the rate of decolorization, which is frequently blocked at high concentrations of toxicant. These models can be used to simulate the process. The best model based on statistical analysis was Teissier with the highest value for the adjusted coefficient of determination and the lowest values for RMSE, AICc and the closest value to 1.0 for accuracy and bias factors. The Teissier 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 BV is toxic and slows down the rate of decolourisation at higher concentrations. The maximum BV specific biodegradation rate (q_{max}), half-saturation concentration (K_S), half inhibition concentration (K_i) was 0.145 h^{-1}, 0.408 mg/L and 73.205 mg/L, respectively.

INTRODUCTION

Although dyes serve an important purpose in the manufacturing and industrial sectors, improper waste management will lead to contamination by dye. Although natural colors have no alternatives, these manufactured colours may be harmful to humans and animals alike. Dye contamination of the main water supply is a direct result of rapid industrialisation, which has negative ecological effects. Colorful, high in BOD (biochemical oxygen demand) and COD (chemical oxygen demand), with high TOC (total organic carbon) and higher SS (suspended solids), temperature (high), pH (low), turbidity (high), and toxicity, these textile effluents are often overlooked [2].

Basic dyes, particularly those having a triphenylmethane structure, are extremely hazardous to fish and can kill them quickly. Many acid dyes appear to be toxic to fish [3,4]. Recent years have seen an explosion in the number of reports of microbes capable of decolorizing triphenylmethane colors at the laboratory scale [3–7]. Basic violet 3 (crystal violet), a commercially-used triphenylmethane textile dye, is a refractory molecule because it is poorly digested by bacteria and, as a result, persists for a long time in the environment [6,7]. Some fish species may be more susceptible to developing tumors when exposed to Basic Violet 3, a compound that acts as a mutagen, mitotic toxin, and clastogen. There is evidence that basic violet 3 causes cancer in rats and mice. Thus, basic violet 3 bioaccumulation raises problems for both the environment and animal health [8–10]. The incorrect disposal of chemical waste from dyeing factories has led to the discovery of basic violet 3 in water streams. the public may be exposed to the dye and its metabolites through the eating of treated chicken products due to its low cost, efficacy as an antifungal agent for commercial poultry feed, and quick availability [8–10].

Contaminants in soil, water, or sediments can be remedied by bioremediation, which makes useful use of biodegradable methods to remove or purify pollutants that otherwise might endanger public health and safety [11,12]. According to this definition, bioremediation is the process of using organisms to remove, bind, or convert environmental pollutants. It is common knowledge in the realm of dyes that certain microbes may degrade the poison in their environment. Microorganisms' potential to break down, decolorize, transform, and mineralize

colors into harmless, non-toxic byproducts has been studied for decades. Further, microorganisms used for color degradation have a positive environmental impact since they require less chemicals to eradicate the polluted area. When compared to the costs of chemical and physical breakdown processes, bioremediation is a more economical option since it requires less energy when fewer chemicals are used.

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 it simple equation and has been reported in several studies [13–15] despite the existence of numerous other secondary models such as Teissier, Aiba, Yano and Koga, Hans-Levenspiel, Webb and the Luong that can predicte concentrations of toxicant that can completely ceased growth or degradationr rate.

In a previous work, a recalcitrant dye; Basic Violet 3 is degraded by *Staphylococcus* aureus and hence has the potential to be a remediation agent. According to this definition, bioremediation is the process of using organisms to remove, bind, or convert environmental pollutants. It is common knowledge in the realm of dyes that certain microbes may degrade the poison in their environment. Microorganisms' potential to break down, decolorize, transform, and mineralize colors into harmless, non-toxic by-products has been studied for decades.

Further, microorganisms used for colour degradation have a positive environmental impact since they require less chemicals to eradicate the polluted area. When compared to the costs of chemical and physical breakdown processes, bioremediation is a more economical option since it requires less energy when fewer chemicals are used. 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 from Figure 8 (Specific growth of *Staphylococcus aureus* in the presence of basic violet 3 and basic green 4 under different initial dye concentration. pH: 7; Initial dye concentration: 10-500 mg/L) [16] were electronically processed using WebPlotDigitizer 2.5 [17] which helps to digitize scanned plots into table of data with good precision and reliability [18,19]. The data then extracted to excel file format for further analysis.

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

Root-mean-square error (RMSE) is a measure of how dispersed the residuals (prediction errors) are relative to the mean. In Eq. 1, p represents the number of parameters in the evaluated model, Obi represents the experimental data, Pdi represents the values predicted by the model, and n represents the quantity of experimental data.

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

Author	Degradation Rate	Author
Monod	$q_{\max}\frac{S}{K_s+S}$	[20]
Haldane	$\frac{q_{\max}\frac{S}{S+K_s+\frac{S^2}{K_i}}}{}$	[21]
Teissier	$q_{\max}\left(1-\exp\left(-\frac{S}{K_i}\right)-\exp\left(\frac{S}{K_s}\right)\right)$	[22]
Aiba	$q_{\max}\frac{S}{K_s+S}\exp(-KP)$	[23]
Yano and Koga	$\frac{q_{\max}S}{S+K_s+\left(\frac{S^2}{K_1}\right)\left(1+\frac{S}{K}\right)}$	[24]
Han and Levenspiel	$q_{\max}\left[1-\left(\frac{S}{S_m}\right)\right]^n\left[\frac{S}{S+K_s\left(1-\frac{S}{S_m}\right)^m}\right]$	[25]
Edward (Webb)	$q_{\max}\frac{S\left(1+\frac{S}{K}\right)}{S+K_s+\left(\frac{S^2}{Ki}\right)}$	[26]
Luong	$q_{\max}\frac{S}{S+K_s}\left[1-\left(\frac{S}{S_m}\right)^n\right]$	[27]

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)

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

This error function penalizes for a high number of parameters; as a general rule, a model with fewer parameters would have a reduced root-mean-squared error (RMSE) [28], which is preferable. The coefficient of determination, or R^2, is commonly used in linear and nonlinear regression for judging the degree of fit. Unfortunately, the approach does not allow for open-ended model comparisons since it does not account for the amount of model parameters. A method is provided below to calculate the quality of nonlinear models using an adjusted R2 that accounts for the number of parameters in the models (Eqns. 2 and 3).

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

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

where

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

An indicator of the relative quality of statistical models is provided by the Akaike information criterion (also known as AIC). Information theory is the foundation upon which it is built. The error function makes a trade-off between the goodness of fit of different models while taking the number of parameters in the model into account. The model that has the lowest value for the AIC metric is the one that should be chosen as the best option. A corrected version of the AIC, known as the Akaike information requirements (AIC) with the correction or AICc, is used in place of the original when the amount of data in a study is limited in relation to the total number of parameters [29].

A difference of five normally suggests that there is a greater possibility of the data with the smaller value being true or correct. However, the actual figures themselves are not significant; what is important is the magnitude of the difference. The formula includes a variables penalty, which states that the greater the AIC value, the less parsimonious the model is. This variable penalty increases as the number of variables increases. When trying to fit experimental data, AIC strongly advises against using more complicated models (also known as "overfitting"). 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

Accuracy Factor (AF) and Bias Factor (BF) (Eqns. 5 and 6) are two more goodness-of-fit models that have been adopted from popular use in predictive microbiology for bacterial growth in food science. Both of these models may be found in Equations 5 and 6 [30]. 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 [1]

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 [1], the normality tests carried out is based on the tests of Kolmogorov-Smirnov [31,32], Wilks-Shapiro [33] and the D'Agostino-Pearson omnibus K2 test [34].

RESULTS AND DISCUSSION

For the purpose of fitting the particular decolorization rate, a number of secondary models (Figs. 1–8) were utilized, and while the most of these models demonstrate visually acceptable fitting (with the exception of Monod), the Hans–Levenspiel model was unable to converge. Teissier was found to be the most accurate model based on statistical analysis; it had the highest value for the adjusted coefficient of determination, the lowest values for RMSE and AICc, and the value that was closest to 1.0 for accuracy and bias factors.

Teissier was the best model. The Teissier model was deemed to be suitable for use in fitting the experimental data since it passed the normality tests and was found to adhere to the model's assumptions. The results of the performed normality tests indicate that the model satisfies the requirements for passing the normalcy tests with a p value greater than 0.05 for each of the normality tests performed [1]. The experimental data obtained indicates that BV is toxic and slows down the rate of decolourisation at higher concentrations. The maximum BV specific biodegradation rate (q_{max}), half-saturation concentration (K_S), half inhibition concentration (K_i) was 0.145 h^{-1}, 0.408 mg/L and 73.205 mg/L, respectively.

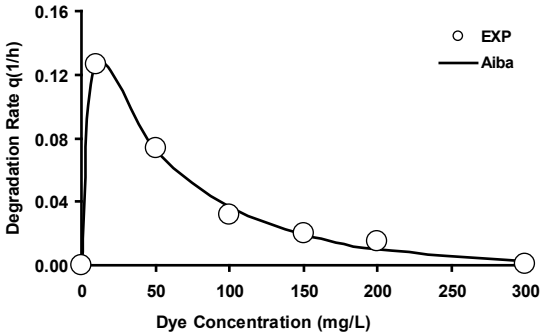

Fig. 1. Fitting the effect of Basic violet 3 dye concentration on specific growth rate by *Staphylococcus aureus* using the Aiba model.

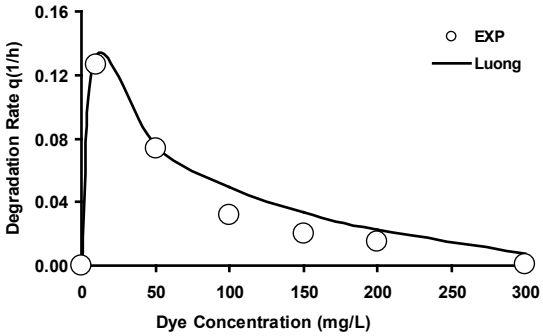

Fig. 2. Fitting the effect of Basic violet 3 dye concentration on specific growth rate by *Staphylococcus aureus* using the Luong model.

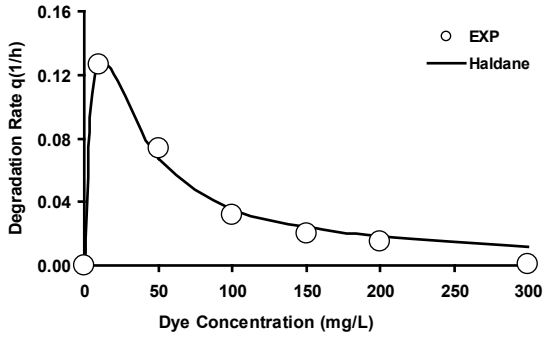

Fig. 3. Fitting the effect of Basic violet 3 dye concentration on specific growth rate by *Staphylococcus aureus* using the Haldane model.

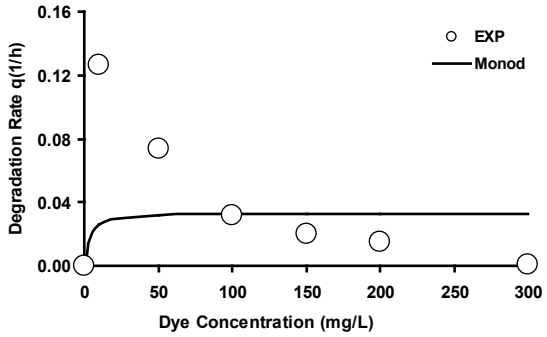

Fig. 4. Fitting the effect of Basic violet 3 dye concentration on specific growth rate by *Staphylococcus aureus* using the Monod model.

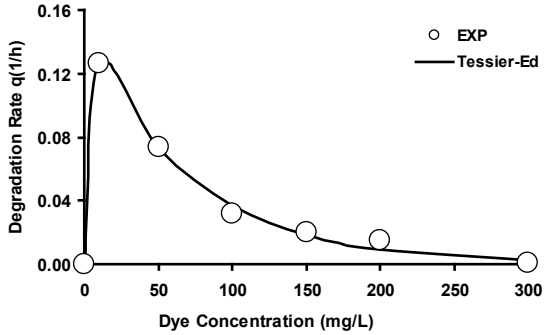

Fig. 5. Fitting the effect of Basic violet 3 dye concentration on specific growth rate by *Staphylococcus aureus* using the Teissier model.

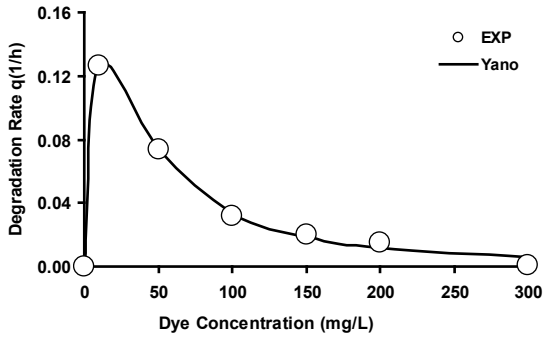

Fig. 6. Fitting the effect of Basic violet 3 dye concentration on specific growth rate by *Staphylococcus aureus* using the Yano model.

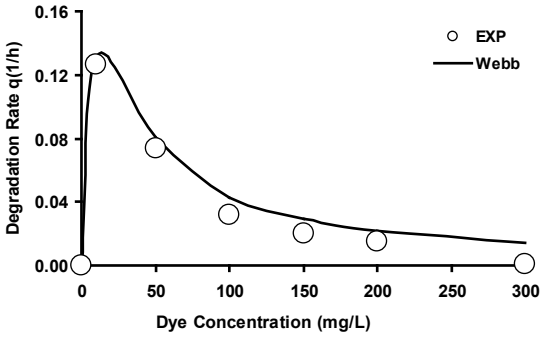

Fig. 7. Fitting the effect of Basic violet 3 dye concentration on specific growth rate by *Staphylococcus aureus* using the Webb model.

Table 2. Statistical analysis of kinetic models.

Model	*p*	RMSE	R^2	adR^2	AICc	BF	AF
Aiba	3	0.003	0.995	0.993	-83.57	1.069	1.196
Luong	4	0.011	0.952	0.904	-48.54	1.460	1.460
Haldane	3	0.006	0.982	0.972	-72.42	1.373	1.402
Monod	2	0.044	0.764	0.665	-43.84	1.297	2.218
Tessier-Edward	3	0.003	0.995	0.993	-83.39	1.052	1.192
Yano	3	0.003	0.997	0.994	-73.17	1.185	1.270
Webb	4	0.010	0.921	0.921	-50.28	1.531	1.270
Hans-Levenspiel	5	0.012	N.A.	N.A.	-50.28	1.5504	1.5504

Note:

df Degree of freedom
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
N.A. Not available

In spite of the fact that this kind of activity is commonly carried out in other xenobiotics-degrading microorganism operations, mathematical modeling on the effect of substrate (dyes) on the growth rate of dye-degrading bacteria is rarely done. The model parameters that are derived from this kind of exercise can be a helpful tool in evaluating the efficiencies of different degraders, and they can also be used to predict the inhibitory effect that substrate has in field experiments. Additionally, the Hans-Levenspiel model was the most accurate representation of *Ralstonia eutropha*'s ability to break down methylene blue (MB). Maximum MB specific biodegradation rate (q_{max}), maximum permissible MB concentration (S_m), and shape factors (n and m) were determined.7.37 mg g_{cell}^{-1} h^{-1}, 32.13 mg/L, 158.8 mg/L, 0.27, and 0.76, respectively [35].

The Haldane model is typically the one that serves as the foundation for the majority of the research conducted on dye decolorizing kinetics. For example, in the process by which Congo red is broken down by Bacillus species, the Haldane model and the Monod model were applied, and it was revealed that the Haldane model was the superior of the two. The Monod model, on the other hand, was shown to be less accurate [36]. 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 [13] and the bio-degradation of Tectilon Yellow 2G (TY2G) by a *Pseudomonas putida* mutant [37].

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 free cell system was 26.30 mg/g/h at an initial MO concentration of 32.7 mg/L [38].

Because it has been established that the Haldane model is more accurate when compared to other models, this generalization regarding the employment of the Haldane model in published studies must be treated with the utmost caution. For example, in addition to the Haldane model, which is the one that is referenced the vast majority of the time, there is also the model that has been developed by others [39], several other different models have been found to be optimal such as Luong [40,41] and Edward [42].

As a direct consequence of this, the utilization of comprehensive models that are easily accessible could consequently replace the use of the Haldane in certain instances. Because it is the only way to truly fit these other models to the data that is available for either the growth or degradation rate, the exclusive utilization of the Haldane model must not be used freely because it is the only way that it can be accomplished. This is due to the fact that it is the only way to truly fit these other models to the data that is available. Following that, the proper statistical analysis needs to be carried out.

CONCLUSION

Because of its antibacterial, antifungal, and antiparasitic properties, basic violet 3 is an essential dye for the prevention and treatment of fish diseases. The use of microorganisms for the purpose of BV bioremediation is becoming increasingly common. A number of secondary models, including Monod, Haldane, Teissier, Aiba, Yano and Koga, Hans-Levenspiel, Webb, and the Luong model, can be used to estimate the rate of decolorization, which is frequently blocked at high concentrations of toxicant. These models can be used to simulate the process. The findings indicate that the majority of the models, with the exception of Monod and Hans -Levenspiel, are capable of providing an adequate fit to the experimental data. The Tessier model was determined to be the most accurate and have the least amount of bias based on statistical analysis. It had the highest value for the adjusted coefficient of determination, the lowest values for RMSE, AICc, HQC, and BIC, and the value that was closest to 1.0 for accuracy and bias factors. In the normality tests, the Teissier model performed as expected, indicating that it is suitable for use in determining how the experimental data should be modeled. The results of the normality tests, which included the Kolmogorov-Smirnov test, the Wilks-Shapiro test, and the D'Agostino-Pearson omnibus K2 test, indicate that the model passes the normality tests with a p value greater than 0.05 for each of the normality tests that were carried out. The experimental data obtained indicates that BV is toxic and slows down the rate of decolourisation at higher concentrations. The maximum BV specific biodegradation rate (q_{max}), half-saturation concentration (K_S), half inhibition concentration (K_i) was 0.145 h^{-1}, 0.408 mg/L and 73.205 mg/L, respectively. The parameters obtained from this exercise can be utilized to model the bioremediation of BV in the future.

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