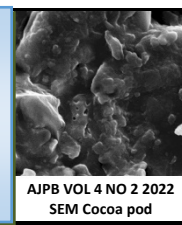


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Degradation Kinetics of Basic Violet 3 by *Staphylococcus aureus*

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ABSTRACT

Synthetic dyes are abundantly used in recent years and mainly consumed in the textile, pharmaceutical, plastic and cosmetic industries. The release of toxic constituents from dyes give adverse effect on human health and marine life. In textile industries, large amount of dye discharged into the wastewater and eventually to the aquatic system are mainly came from the critical step of dyeing and finishing processes in textile. The be able to precisely forecast the rate of bioremediation, depends on the gathering of precise rate of decolourisation, and this can be inaccurately acquired by natural logarithm transformation of the decolourisation process over time. In cases like this, a nonlinear regression of the curve must be performed making use of accessible rate models. Consequently, numerous primary models for example modified Logistic, modified Gompertz, modified logistics, modified Richards, modified Schnute, Baranyi-Roberts, Buchanan-3-phase, von Bertalanffy and the Huang models were utilized to fit the specific decolourisation rate. Several models did not converge and was disregarded and only Huang, Baranyi-Roberts, modified Gompertz, modified Richards and modified Logistics could actually model the data. The very best model according to statistical analysis was Baranyi Roberts with the highest value for 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 Baranyi-Roberts fitted curve was discovered to conform to normality tests and is satisfactory to be used to fit the experimental data. The parameters extracted from this exercise may be used for additional secondary modelling training to glean information about how substrate (dye) impact the rate of decolourisation of the substrate.

INTRODUCTION

As dye is coloured components or pigments, it is easily to contaminate the water. With a very small amount of dye in water in range of 10 mg/L to 50 mg/L, it is quietly visible and affects the water transparency, aesthetic value, and gas solubility of water bodies [1]. It also prevents the effective light penetration into the aquatic system and disrupt the development of aquatic life. Basic dyes, due to their triphenylmethane structure, are extremely toxic to fish and other aquatic organisms. There are many beneficial microorganisms that can decolorize triphenylmethane dyes, and this ability has been known for decades. Crystal violet, or basic violet 3 (Gentian Violet or Methyl Violet 10B), is a type of dye made from triphenylmethane.

The dye has a number of histology applications, including Gram staining for bacterial identification. Crystal violet is a safe alternative to fluorescent, intercalating dyes like ethidium bromide for staining DNA during DNA gel electrophoresis. It can be used either by adding it to the agarose gel before electrophoresis or by applying it to the gel afterward. When used at a concentration of 0.001% and allowed to stain a gel for 30 minutes following electrophoresis, it is sensitive enough to detect DNA at concentrations as low as 16 ng. Sensitivity can be increased to 8 ng of DNA by the use of a methyl orange counterstain and a more intricate staining procedure. Crystal violet has become a popular alternative to fluorescent stains because it allows DNA cloning to be performed in vitro without the need for ultraviolet irradiation, which can cause DNA damage [2,3].

As a commercial textile dye, its use is extensive. It is a xenobiotic compound that resists biodegradation, so it stays in the environment for a longer period of time. Water takes on a violet hue even at low concentrations thanks to a stubborn molecule called basic violet 3. For this reason, the violet-colored waste products of its production or consumption cannot be released into the environment. The fact that Basic violet 3 is a mutagen, a mitotic poison, and a potent clastogen raises further concerns about its role in promoting tumor growth in certain fish species [4]. Basic violet 3 has been shown to cause cancer in rodents and mice [5,6]. As a result, basic violet 3 bioaccumulation raises concerns for both ecosystems and animal populations.

Adsorption, physical precipitation and flocculation, reverse assimilation, compound oxidation and reduction, electrochemical treatment, photolysis, accelerated oxidation, and substance corruption are just some of the processes that have been considered for the elimination of Basic violet 3 from wastewater. Bioremediation is a low-cost approach, has low environmental impact, and low sludge production, microbes have garnered a lot of support for their use in decolorizing and degrading dyes and pigments. There is now a lot of research going on to figure out what kind of microbial biomass is the most successful and cost-effective at getting rid of dyes from vast quantities of polluted water [7].

Biological decolorization has been studied as a means to alter, degrade, or mineralize colors throughout the past few decades. Decolorization and degradation methods like this offer an inexpensive and environmentally friendly substitute to chemical decomposition operations. The treatment of various natural effluents and color effluents through biodegradation is a notable breakthrough in wastewater management. To understand the degradation mechanism in these organisms, precise assignment of degradation kinetics and modeling are urgently required.

The use of a linearized version of a curve that is obviously nonlinear is widely documented in the literature. The drawback is that it is more difficult to quantify uncertainty, which is commonly expressed as a 95% confidence interval, when nonlinear data is translated into a linear format since the error structure of the data is changed [8]. The main objective of this research is to model the degradation or the decolorization of Basic Violet 3 dye using non-linear regression models which include modified Gompertz, modified Logistic, modified Richards, Baranyi-Roberts, modified Schnute, von Bertalanffy and the Huang models. A more accurate parameters of the decolorization process can be obtained using the best model.

MATERIALS AND METHODS

Data acquisition

Data was obtained from a published work [9] from Figure 1 and were electronically processed and redrawn using the software WebPlotDigitizer 2.5 [10] which aids in the accurate and reliable digitization of scanned plots into tables of data [11,12].

Fitting of the data

In this research, we used CurveExpert Professional (Version 1.6), software that minimizes the sums of squares of the differences between predicted and measured values. The program employs a Marquardt algorithm (Table 1).

Table 1. Mathematical models governing decolorization rate used in this study.

Model	p	Equation
Modified Logistic	3	$y = \frac{A}{1 + \exp\left[\frac{4q_m}{A}(\lambda - t) + 2\right]}$
Modified Gompertz	3	$y = A \exp\left\{-\exp\left[\frac{q_m}{A}(\lambda - t) + 1\right]\right\}$
Modified Richards	4	$y = A \left\{1 + v \exp(1 + v) \exp\left[\frac{q_m}{A}(1 + v) \left(1 + \frac{1}{v}\right)(\lambda - t)\right]\right\}^{\left(\frac{-1}{v}\right)}$
Modified Schnute	4	$y = \left(q_m \frac{(1 - \beta)}{\alpha}\right) \left[\frac{1 - \beta \exp(\alpha\lambda + 1 - \beta - \alpha t)}{1 - \beta}\right]^{\frac{1}{\beta}}$
Baranyi-Roberts	4	$y = A + q_m x + \frac{1}{q_m} \ln\left(\frac{e^{-q_m x} + e^{-h_0} - e^{-q_m x - h_0}}{e^{-q_m x} + \frac{1}{\mu_m} \ln\left[\frac{e^{-q_m x} + e^{-h_0} - e^{-q_m x - h_0}}{e^{-q_m x} - A}\right] - 1}\right)$
Von Bertalanffy	3	$y = K \left[1 - \left[1 - \left(\frac{A}{K}\right)^3\right] \exp\left\{-\left(\frac{A}{K}\right)^3 t\right\}\right]^{\frac{1}{3}}$
Huang	4	$y = A + y_{\max} - \ln(e^A + (e^{y_{\max}} - e^A)e^{-q_m B(x)})$ $B(x) = x + \frac{1}{\alpha} \ln \frac{1 + e^{-\alpha(x-\lambda)}}{1 + e^{\alpha\lambda}}$
Buchanan Three-phase linear model	3	$Y = A, \text{ IF } X < \text{LAG}$ $Y = A + K(X - \lambda), \text{ IF } \lambda \leq X \leq X_{\max}$ $Y = Y_{\max}, \text{ IF } X \geq X_{\max}$

Note:

A= Decolorization lower asymptote;

μ_m = maximum specific decolorization rate;

v= affects near which asymptote maximum decolorization occurs.

λ =lag time

$y_{\max}$ = Decolorization upper asymptote;

e = exponent (2.718281828)

t = sampling time

α, β, k = curve fitting parameters

h_0 = a dimensionless parameter quantifying the initial physiological state of the reduction process.

The lag time (h^{-1}) or (d^{-1}) can be calculated as $h_0 = q_m \lambda$

Statistical analysis

Extensive error function analyses were utilized in this study and include Root-mean-square error (RMSE), and Ross's bias factor (BF), and accuracy factor (AF) and adjusted coefficient of determination ($\text{adj}R^2$) [13]. The rootmean-square error or RMSE was calculated according to Eq. 1;

The RMSE was calculated as follows,

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

Where,

n = number of experimental data

Pd_i = predicted values by the model

Ob_i = experimental data

p = parameters number of the model

As general rule, those model that has smaller number of parameter corresponds in smaller RMSE value [14]. Determining R^2 , also known as the coefficient of determination, because it does not take into account the number of parameters of models, an alternative approach is to use an adjusted form of R^2 that has been modified to account for the large number of model parameters (Eqns. 2 and 3) of which it is used to work out the quality of nonlinear models according to the formula below;

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

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

Where,

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

Information theory forms the basis of the Akaike information criterion (AIC). The method uses a minimal AIC value as its criterion of choice. In many cases, this value is undesirable; for instance, an AICc value of -10 is preferred to a main value of -1. The formula takes into account a variable penalty, with a higher AIC value indicating a less parsimonious model the more variables there are. When fitting experimental data, AIC warns against employing more intricate models. When the number of parameters in a study is low, researchers often turn to a modified version of AIC called corrected AICc. [15]. AICc is calculated using the following equation (Eqn. 4);

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

Where,

n number of data points
 p parameter numbers of the model

When calculating AICc, the degree to which a model has changed is taken into account. The model also accounts for the fact that different models have different numbers of parameters. Models with smaller AICc values are more likely to be correct when trying to interpret the results [16]. Accuracy Factor (AF) and Bias Factor (BF) (Eqns. 5 and 6) are another goodness-of-fit of models adapted from prevalent use in predicted microbiology for bacterial growth in food science [17]. A perfect correlation between experimental and predicted values is determined by the statistics. A fail-safe model has a BF greater than 1.0, while a fail-dangerous one has a BF less than 1.0. On the other hand, the AF is always less than one, with values close to one predicted by the most accurate models.

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

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

Residual's assesment of normality (Eqn. 7) was carried out using the software GraphPad Prism® 6 (Version 6.0, GraphPad Software, Inc., USA). Residual is mathematically represented as follows;

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

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 [18].

RESULT AND DISCUSSION

The specific decolourisation rate was obtained from a primary modelling exercise using various models (Figs. 1 to 5), which shows visually acceptable fitting. Several models such as failed

to converge and was omitted. One of the models, the modified Gompertz is very popular and has been used to model growth curve on xenobiotics including dyes as substrates [19–21]. The other models are rarely used in modelling dye degradation or decolorization. Only Huang, Baranyi-Roberts, modified Gompertz, modified Richards and modified Logistics were able to model the data whilst other models failed to converge and were omitted. The best model based on statistical analysis was Baranyi Roberts. The Baranyi-Roberts model has found numerous utility in modelling microorganisms growth in food and other applications including biodegradation of toxicants [22–28]. The Baranyi-Roberts fitted curve was found to conform to normality tests and is adequate to be used to fit the experimental data. The model has highest value for 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.

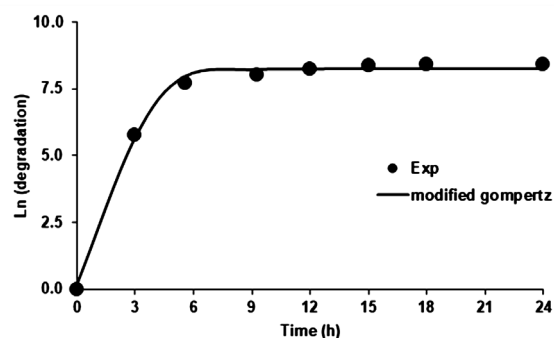


Fig. 1. Fitting the effect of Basic Violet 4 (●) dye on the decolourisation rate using the modified Gompertz model by *Staphylococcus aureus*.

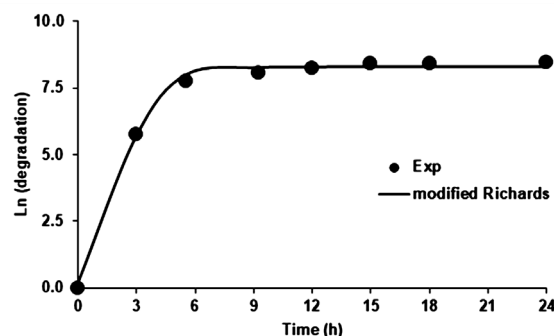


Fig. 2. Fitting the effect of Basic Violet 4 (●) dye on decolourisation rate experimental data with Modified Richards model by *Staphylococcus aureus*.

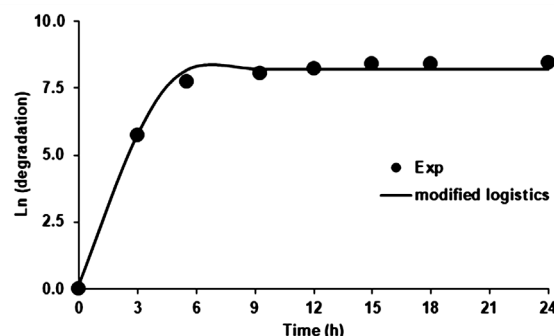


Fig. 3. Fitting the effect of Basic Violet 4 (●) dye on decolourisation rate experimental data with Modified Logistics model by *Staphylococcus aureus*.

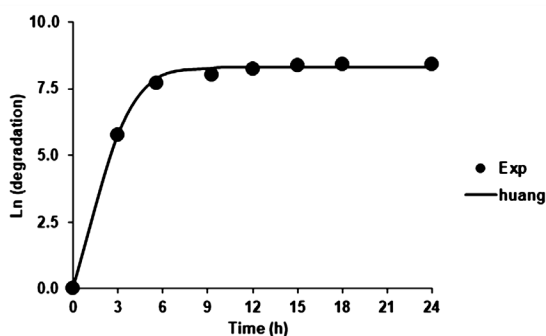


Fig. 4. Fitting the effect of Basic Violet 4 (●) dye on decolourisation rate experimental data with Huang model by *Staphylococcus aureus*.

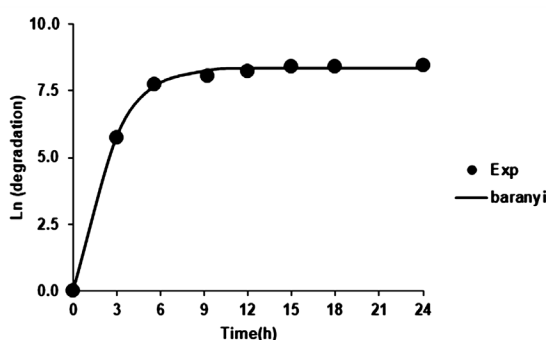


Fig. 5. Fitting the effect of Basic Violet 4 (●) dye on decolourisation rate experimental data with Baranyi model by *Staphylococcus aureus*.

Table 2. Statistical analysis of kinetic models.

model	p	RMSE	AdjR ²	AICC	BF	HQC	BIC	AF
Huang	4	0.1687	0.995	13.983	1.005	-28.16	-25.69	1.01
Baranyi-Roberts	4	0.13	0.997	9.888	1.001	-32.25	-29.793	1.008
Modified Gompertz	3	0.243	0.988	75.523	1.001	-23.15	-20.079	1.013
Modified Richards	4	0.243	0.988	75.525	1.001	-23.154	-20.077	1.01
Modified Logistics	3	0.291	0.985	22.749	1.003	-19.394	-16.933	1.016

Note:

p no of parameter
 SSE Sums of Squared Errors
 RMSE Root Mean Squared Error
 R² Coefficient of Determination
 adjR² Adjusted Coefficient of Determination
 AICC Corrected Akaike Information Criterion
 BF Bias Factor
 AF Accuracy Factor

An important aspect of this study is the modelling exercise yield important decolourisation rate parameters that can be further utilized to gleam on the effect of substrate (dye) to the decolourization rate. The normality tests carried out is based on the tests of Kolmogorov-Smirnov [29,30], Wilks-Shapiro [31] and the D'Agostino-Pearson omnibus K2 test [32] were utilized to the residuals from the Baranyi-Roberts model and were found to pass the normality tests with $p > 0.05$ for all normality tests carried out [18].

The modelling on the effect of time on decolourisation is rarely reported in the literature where researchers often transforming the decolourisation profile into a linear form to obtain the specific decolourization rate [33]. Hence the best

model in this study— the Baranyi-roberts model being the best model to fit the nonlinear curves of dye decolourisation is novel to the best of our knowledge. Transforming an otherwise nonlinear curve into a linearized form disrupt the error structure and must be avoided [16]. In addition, the use of mathematical models allow another important parameter; the lag time to be obtained [34]. Often growth on toxic xenobiotics including dye at high concentration increases the lag period as the cells try to offset the toxicity through various intrinsic mechanisms such as pumping, producing metabolites that can sequester the xenobiotics or often enzymes that can degrade the xenobiotics [35–40]. All of these activities require expenditure of energy that is translated as an increase in lag period [41]. The results will be very important for future bioremediation works carried out in the field.

CONCLUSION

Numerous primary models for example modified Logistic, modified Gompertz, modified logistics, modified Richards, modified Schnute, Baranyi-Roberts, Buchanan-3-phase, von Bertalanffy and the Huang models were utilized to fit the specific decolourisation rate of Basic Violet 3 by a bacterium. Of these models some did not converge and was disregarded and only Huang, Baranyi-Roberts, modified Gompertz, modified Richards and modified Logistics could actually model the data. The very best model according to statistical analysis was Baranyi Roberts with the highest value for 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 fitted curve from the Baranyi-Roberts model conform to normality tests. Current works include secondary modelling training to gather info on how the concentration of the substrate (dye) inhibits the rate of decolourisation of the substrate.

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