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Modelling the Decolorization of Malachite Green by *Staphylococcus aureus*

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ABSTRACT

Dyes plays an important role in our everyday life. From manufacturing plastics, paints, textile and even pills contain traces of dye used. With the ever-increasing demand for dye with growing world populations, the use of synthetic dyes has grown linearly. 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 inaccurately obtained by natural logarithm transformation of the decolourisation process over time. In this instance, a nonlinear regression of the curve needs to be carried out utilising available rate models. Hence, various primary models such as 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 failed to converge and was omitted and only Huang, Baranyi-Roberts, modified Gompertz, modified Richards and modified Logistics were able to model the data while other models failed to converge and were omitted. The best model based on 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 found to conform to normality tests and is adequate to be used to fit the experimental data. The parameters obtained from this exercise can be utilized for further secondary modelling exercise to gleam information on how substrate (dye) affect the rate of decolourisation of the substrate.

INTRODUCTION

Malachite green has been widely used in the aquaculture industries as a topical treatment through bath or flush methods, with little consideration given to the possibility that such therapeutants may also be absorbed systemically and produce significant internal effects. It is also used as a dye in the silk, paper, wool, jute, cotton, leather, and acrylic industries, as well as a food coloring agent, medical disinfectant, food additive, and anthelminthic. In addition to its widespread application in the textile industry, Malachite Green (MG), a member of the triphenylmethane dye, is used as a fungicide and an ectoparasiticide in aquaculture. Even though MG's effects on aquatic invertebrates and algae are still being investigated, the dye and its derivatives are known to accumulate in aquaculture products like fish, prawn, and crab, and are widely reported to be toxic to many species of fish at concentrations as low as 1 mg/L. It's also carcinogenic and genotoxic, so it could be bad for people's health [1–4]. Leucomalachite green, a reduced form of malachite green used to treat and prevent fungal and parasitic infections, builds up in the tissues of exposed fish. Serum, kidney, liver, skin, muscle, and viscera of a wide range of experimental animals, including fish, are the primary storage sites for this protein [5–7].

Because of this, many countries have banned the use of this dye. This includes the European Union, the United States, and others. However, MG is still used in some parts of the world because it is highly efficient, cheap, and easily accessible. Because it is so accessible, there is concern that it will be used illegally. It is used in the United States to treat illnesses in tropical fish. It has been suggested that MG is used in Asian countries as

a means of treating external parasites and fungal infections in fish aquaculture. However, when compared to the removal of other contaminants, the removal of MG from aquaculture effluent has received little to no attention. Therefore, MG contamination in aquaculture effluent is possible, which could have serious consequences for the ecosystem [8–11]. There are several different forms of malachite green on the market, the most common of which is a 50 percent solution of the oxalate or hydrochloride salt. Malachite green hydrochloride, an industrial grade variety, is precipitated during production as a double zinc salt via the addition of zinc chloride. Like other triphenylmethanes, this dye has the ability to exist in both the dye salt and the carbinol or pseudobase ionic forms. Due to their much higher lipid solubility as the pseudobase, these ions are likely to enter cells in this form [12].

Bioremediation is the productive use of the biodegradative process to remove or detoxify pollutants enter the environment and threaten the public health or safety of the environment usually as contaminants of soil, water or sediments [13,14]. In this context, bioremediation is the use of microorganism to degrade, sequester or conjugate environmental pollutants. Certain microorganisms have the capability to degrade contaminant in the environment, which has been well established in dyes field. Over the past decades, the ability of microorganism has been investigated as a method to degrade, decolourise, transform and total mineralisation of dyes to safe, non-toxic by-products. Furthermore, the role of microbes used for dye degradation is environmentally friendly as less chemical is used to clear the contaminated site. Besides, when less chemical is involved, lower energy is required for the bioremediation process, thus making it a cost-effective alternative to both chemical and physical decomposition process.

The objective of this research is to model the degradation or the decolourization of Malachite Green dye by a bacterium using non-linear regression such as modified Logistic, modified Gompertz, modified logistics, modified Richards, modified Schnute, Baranyi-Roberts, Buchanan-3-phase, von Bertalanffy and the Huang models. This modelling will allow for more accurate parameters of decolourization to be obtained. The best model will be evaluated based on various statistical test such as the adjusted coefficient of determination ($adjR^2$), root mean square error (RMSE), corrected Akaike Information Criterion (AICc), Hannan-Quinn Information Criterion (HQC), Bayesian Information Criterion (BIC), accuracy factor (AF) and bias factor (BF).

MATERIALS AND METHODS

Data acquisition

Graphical data of a published work [15] from Figure 1 were electronically processed using WebPlotDigitizer 2.5 [16] which helps to digitize scanned plots into a table of data with good precision and reliability [17,18].

Fitting of the data

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

Statistical analysis

The root mean-square error or RMSE was calculated according to **Eq. 1**, where p is the number of parameters of the assessed model, Ob_i are 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,

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

where

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

As a general rule, those model that has a smaller number of the parameter corresponds in smaller RMSE value [19].

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

Model	p	Equation
Modified Logistic	3	$y = \frac{A}{\left\{1+\exp\left[\frac{4q_m}{A}(\lambda - t)+2\right]\right\}}$
Modified Gompertz	3	$y = A\exp\left\{-\exp\left[\frac{q_m e}{A}(\lambda - t)+1\right]\right\}$
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	$y = A + q_m x + \frac{1}{q_m}\ln\left(e^{-q_m x}+e^{-h_0}-e^{-q_m x-h_o}\right) - \ln\left(1+\frac{e^{q_m x+\frac{1}{\mu_m}\ln\left(e^{-q_m x}+e^{-h_0}-e^{-q_m x-h_0}\right)}-1}{e^{(y_{max}-A)}}\right)$
Von Bertalanffy	3	$y = K\left[1-\left[1-\left(\frac{A}{K}\right)^3\right]\exp^{-\left(q_m x/3K^{\frac{1}{3}}\right)}\right]^3$
Huang	4	$y = A + y_{\max} - \ln\left(e^A + \left(e^{Y_{max}} - e^A\right)e^{-q_m B(x)}\right)$ $B(x) = x + \frac{1}{\alpha}\ln\frac{1+e^{-\alpha(x-\lambda)}}{1+e^{\alpha\lambda}}$
Buchanan Three-phase linear model	3	Y = A, IF X < LAG Y=A + K(X−λ), IF λ ≤ X ≥ X_{MAX} Y = Y_{MAX}, IF X ≥ X_{MAX}

Note:
A= Decolorization lower asymptote;
μ_m= maximum specific decolorizarion 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_{max}

R^2 is commonly used as a measure of goodness of fit in both linear and nonlinear regression. However, the method does not provide unrestricted comparative analysis due to its disregard for model parameter counts. The quality of nonlinear models can be calculated using the following formula, which utilizes an adjustedR^2 that accounts for the number of parameters in the models (Eqns. 2 and 3).

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

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

where

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

Based on information theory, the Akaike information criterion (AIC) evaluates models according to how well they fit data while also accounting for their level of complexity. When making a model selection, the one with the smallest AIC value wins. When the number of parameters in the study is low, a modified version of AIC called Akaike information requirements (AICc) is used [20]. A delta or difference of 5 indicates that the data with the smaller value is more likely to be accurate or correct, but the actual values themselves are unimportant. As the number of variables increases, the AIC value rises, indicating that the model is less parsimonious. When fitting experimental data, AIC discourages the use of more complex models (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

Commonly used in predicted microbiology for bacterial growth in food science, the Accuracy Factor (AF) and Bias Factor (BF) (Eqns. 5 and 6) are another goodness-of-fit of models [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]

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

Decolourization kinetics

Various primary models (**Fig. 1**) were utilized to fit the specific decolourisation rate, and most of them show 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 [27–29]. 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 while other models failed to converge and were omitted. The best model based on 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 found to conform to normality tests and is adequate to be used to fit the experimental data (Data not shown). The normality tests carried out shows that the model pass the normality tests with p >0.05 for all normality tests carried out [22]. Biodegradation of toxicants is just one of several situations aside from its common usage in food mcirobiology, where the Baranyi-Roberts model has proven useful for modeling microbial growth [30–36].

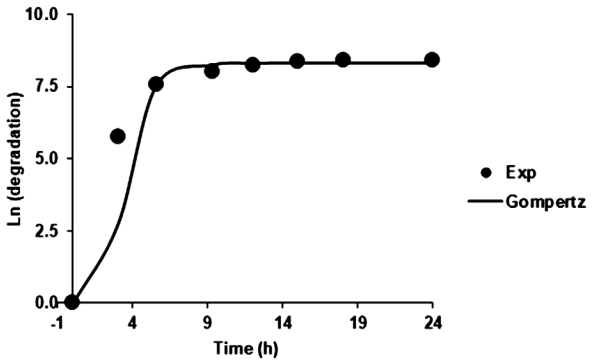

Fig. 1. Fitting the effect of Malachite Green (●) dye on the decolourisation rate experimental data by *Staphylococcus aureus* with the modified Gompertz model (-).

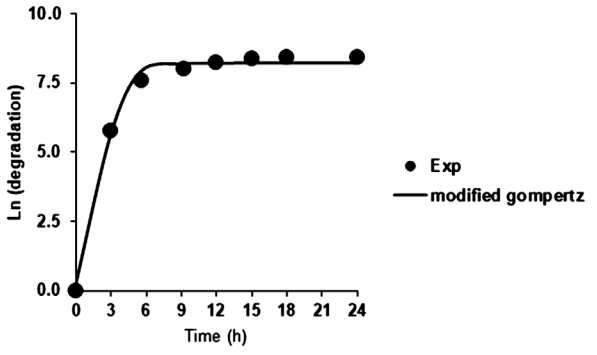

Fig. 2. Fitting the effect of Malachite Green (●) dye on decolurisation rate experimental data with Modified Gompertz model (-) by *Staphylococcus aureus*.

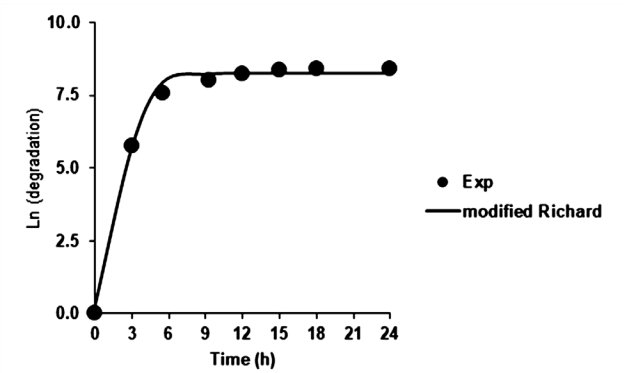

Fig. 3. Fitting the effect of Malachite Green (●) dye on decolurisation rate experimental data with Modified Richards model (-) by *Staphylococcus aureus.*

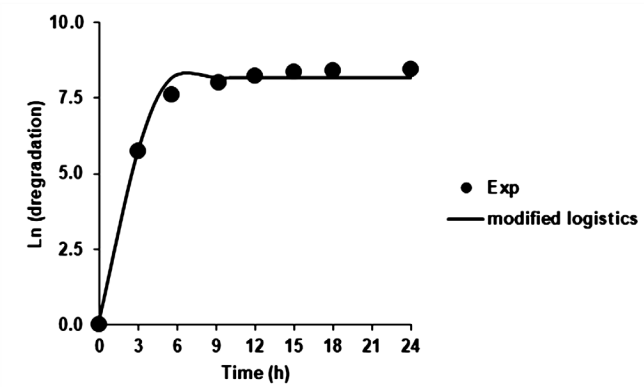

Fig. 4. Fitting the effect of Malachite Green (●) dye on decolourisation rate experimental data with Modified Logistics model (-) by *Staphylococcus aureus.*

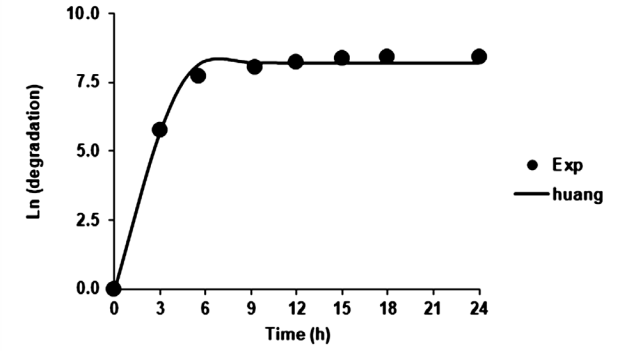

Fig. 5. Fitting the effect of Malachite Green (●) dye on decolourisation rate experimental data with Huong model (-) by *Staphylococcus aureus.*

Important decolourisation rate parameters obtained in this study can be further utilized to gleam on the effect of substrate (Malachite Green) to the decolourization rate. It is observed from the literature search that the modelling on the effect of time on degradation or decolourisation rate for dye is rarely reported in the literature. Most often than not, these researchers transform the decolourisation profile into a linear form through the use of transformation method such as natural logarithm transformation to obtain the specific decolourization rate [37].

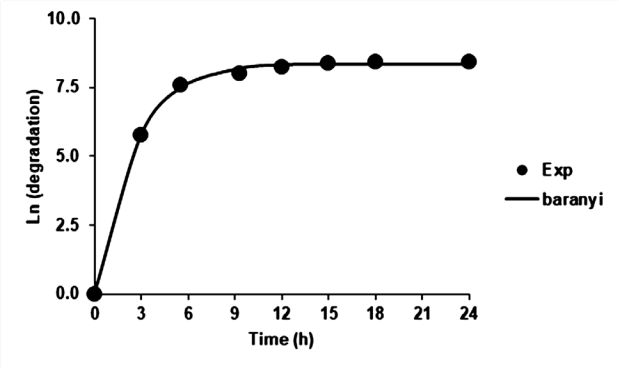

Fig. 6. Fitting the effect of Malachite Green (●) dye on decolourisation rate experimental data with Baranyi model (-) by *Staphylococcus aureus.*

Table 2. Statistical analysis of kinetic models.

model	p	RMSE	AdjR^2	AICc	BF	HQC	BIC	AF
Huang	4	0.325	0.982	24.51	1	-17.624	-15.163	1.019
Baranyi-Roberts	4	0.116	0.997	8.0381	1	-34.105	-31.644	1.007
Modified Gompertz	3	0.248	0.989	20.191	1.002	-21.951	-19.49	1.015
Modified Richards	4	0.4049	0.969	83.687	0.993	-14.991	-11.915	1.025
Modified Logistics	3	0.388	0.971	83.04	1.004	-15.638	-12.562	1.019

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

Transforming an otherwise nonlinear curve into a linearized form disrupt the error structure and must be avoided [38,39]. To the best of our knowledge the Baranyi-Roberts model being the best model to fit the nonlinear curves of dye decolourisation is novel. In addition, the use of mathematical models allow another important parameter; the lag time to be obtained [40,41].

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 [42–44]. All of these activities require the expenditure of energy that is translated as an increase in the lag period [45]. Future bioremediation works will need to rely on mathematical modelling results such as in this study to improve remediation works.

CONCLUSION

Malachite Green is a toxic dye often used in aquaculture. Its bioremediation by a bacterium has been reported. The ability to accurately predict the rate of bioremediation using this bacterium relies upon the gathering of the accurate rate of decolourisation and a nonlinear regression of the curve needs to be carried out utilising available rate models. Hence, various primary models such as 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 failed to converge and were omitted and only Huang, Baranyi-Roberts, modified Gompertz, modified Richards and modified Logistics were able to model the data while other models failed to converge and were omitted. The best model based on 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 found to conform to normality tests and is adequate to be used to fit the experimental data. The parameters obtained from the Baranyi-Roberts model such as the maximum specific decolourisation rate is currently being used for secondary modelling of the effect of dye concentration on decolourization rate.

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