Mathematical Modelling of the Growth of *Bacillus cereus* Strain wwcp1on Malachite Green Dye

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ABSTRACT

In this paper, various growth models such as Von Bertalanffy, Huang, Baranyi-Roberts, Modified Gompertz, Buchnam-3-phase, Modified-Richards and Modified-Logistics, were presented in fitting and evaluating the growth of *Bacillus cereus* wwcp1 on Malachite green dye. The Von Bertalanffy model was found to be the best model with the lowest RMSE and highest R2 values. The Accuracy and Bias factor values were near unity (1.0). The von Bertalanffy parameters such as A (lower asymptote bacterial growth), μ (bacterial growth rate) and k (curve fitting parameter) were found to be 2.757 (95% confidence interval from 2.131 to 3.382), 0.287 (95% confidence interval from 0.244 to 0.329) and 4.323 (95% confidence interval from 4.285 to 4.361) respectively.

INTRODUCTION

A common biocide in the aquaculture industry is malachite green (MG), which is one of the most widely used (MG). One of the most effective anti-protozoal and anti-fungal drugs now available on the market. It has been used to manage skin and gill flukes as a general ectoparasiticide, as well as other parasites. Despite the fact that MG has long been used extensively as a topical therapeutic, aquaculture enterprises have largely neglected the possibility that topically applied therapeutants could have major internal impacts on their products. It is also employed as a food colouring agent and as an ingredient in foods, as a medical disinfectant and anti-helminthic, and as a dye in the textile industry, among other things (such as silk and wool). Because of the dangers it causes to those who consume seafood that has been treated with MG, this material has become extremely contentious [1–4]. MG is produced by condensing benzaldehyde with two parts of diemethylaniline in the presence of strong sulfuric acid or zinc chloride to form a dark green crystalline solid known as MG. It is also possible to obtain MG in a 50/50 solution of acetate and hydrochloride salts, in addition to the salts of oxalate or hydrochloride. MG hydrochloride is manufactured by adding zinc chloride to the mixture and allowing it to precipitate, resulting in the development of a double zinc salt. In addition to the dye salt, a triphenylemethane dye can exist in two more ionic forms: the carbinol or pseudobase. They may enter cells as the pseudobase due to their high lipid solubility and ability to dissolve in lipids MG is reduced to its colourless form, leucoMG, in animals, which is then retained in their tissues as a result of biotransformation. The dye's precursor, leucoMG, has a structural resemblance to classic aromatic amines, and it is possible that it is present in the dye as a contaminant in the commercially manufactured dye. A number of researchers have estimated the LC_{50} values of different commercial dyes in fish at various time points over the course of several studies.

There are numerous variables that influence the toxicity of certain toxicants, such as the temperature, pH, and hardness of the test water, making it difficult to analyse the effects on diverse species of fish [5–9]. The question of whether MG is teratogenic to fish has been investigated in great detail. Many other research, both clinical and experimental, have been conducted on MG over the years. Its toxicity to diverse organisms, aquatic and terrestrial has been reported [5,7–15] and its removal is thus warranted. Biodegradation of MG has been touted as an important aspect of MG bioremediation. The degradation and mineralization of MG by microorganisms has been reported. Bacterial growth and degradation of MG has been studied and show promising results for its potential use for the remediation of MG from the environment [16–28].

Bacterial growth curves typically followed a sigmoidal pattern, beginning with the lag section just after t = 0, followed by the logarithmic section, then the bacteria reach the stationary phase, and finally enter the death phase. Various sigmoidal functions such as Huang, Baranyi-Roberts, Von Bertalanffy, Modified Gompertz, Modified-Logistics, Modified-Richards, Morgan-Mercer-Flodin and Buchnam-3-phase were statistically compared in order to describe the bacterial growth curve [29–33]. The F and t tests were used. The F test compares the lack of fit of the models to the measuring error. While in the t test, confidence intervals for parameters can be estimated and used to differentiate between models. Also, the models were compared in relation to their ease of use.

All sigmoidal functions were modified to include all biologically significant parameters. The maximum specific growth rate (µmax), the lag period, and the asymptotic values are important growth curve parameters. The maximum growth rate (µmax) value can be used in the development of secondary models to investigate the effects of substrate, temperature, pH, and product on growth rate. Most models of bacterial growth, fall somewhere between mechanistic and empirical properties, though these two groups can coexist in reality [33–36]. The use of primary models in the modeling of *Bacillus cereus* wwcp1 growth curve on malachite green dye is presented for the first time in this finding. This study is aimed at evaluating various available mathematical models such as Logistic, Gompertz, Von Bertalanffy, Buchanan three-phase, and, more recently, the Huang model in fitting and analyzing *Bacillus cereus* growth on malachite green dye.

MATERIALS AND METHODS

Data Acquisition
The graphical data from a published work by Wanyonyi *et al* [37] from Fig 5 (effect of temperature on the decolorization of MG dye) was processed using the software Webplotdigitizer 2.5, which digitizes the scanned figure and has been used and recognized by many researchers due to its precision and reliability [38].

Statistical Analysis
As previously, the adjusted coefficient of determination (R^2), accuracy factor (AF), bias factor (BF), Root-Mean-Square Error (RMSE), and corrected AICc (Akaike Information Criterion) were used to calculate the statistically significant difference between the models [29,39–42].

Fitting of the Data
CurveExpert Professional software (Version 1.6) was used to fit the bacterial growth curve using multiple growth models (Table 1) through nonlinear regression with the Marquardt algorithm. The steepest ascent rifle of the curve was used to estimate the highest growth rate (µmax), while the line crossing the X-axis was used to estimate the lag time (λ). For the modeling exercise, the highest growth rate was chosen.

Table 1. Growth models used in this study.

Model	F	Equation
Modified Logistic	3	$y=\dfrac{A}{\left\{1+\exp\left[\dfrac{4\mu_m}{A}(\lambda-t)+2\right]\right\}}$
Modified-Gompertz	3	$y=A\exp\left\{-\exp\left[\dfrac{\mu_m e}{A}(\lambda-t)+1\right]\right\}$
Modified-Richards	4	$y=A\left\{1+v\exp(1+v)\exp\left[\dfrac{\mu_m}{A}(1+v)\left(1+\dfrac{1}{v}\right)(\lambda-t\right.\right.$
Baranyi-Roberts	4	$y = A + \mu_m x + \dfrac{1}{\mu_m}\ln\left(e^{-\mu_m x} + e^{-h_0} - \right.$ $-\ln\left[1+\dfrac{e^{\mu_m x+\frac{1}{\mu_m}\ln\left(e^{-\mu_m x}+e^{-h_0}-e^{-\mu_m x-h_0}\right)}-1}{e^{(y_{max}-A)}}\right]$
Von Bertalanffy	3	$y=K\left[1-\left[1-\left(\dfrac{A}{K}\right)^3\right]\exp\left(-\left(\mu_m x/3K^{\frac{1}{3}}\right)\right)\right]^3$
Huang	4	$y=A+y_{max}-\ln\left(e^A+\left(e^{Y_{max}}-e^A\right)e^{-\mu_m B(x}\right.$ $B(x)=x+\dfrac{1}{\alpha}\ln\dfrac{1+e^{-\alpha(x-\lambda)}}{1+e^{\alpha\lambda}}$
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= Bacterial growth lower asymptote;
µmax= maximum specific bacterial growth rate;
v= affects near which asymptote maximum growth occurs.
λ=lag time
ymax= Bacterial growth upper asymptote;
e = exponent (2.718281828)
t = sampling time
α,β, k = curve fitting parameters
h_0 = a dimensionless parameter that quantifies the initial physiological state of the reduction process.
The lag time (h^{-1}) can be calculated as $h_0=\mu_{max}$

RESULTS AND DISCUSSION

Based on bacterial growth modeling, the best model was discovered to be the von Bertalanffy model, which had the lowest RMSE, AICc, and adjusted R^2 values. The AF and BF values for the model were also outstanding, with values close to 1.0. All of the model fitted to the date with the exception of the Buchanan-3-phase, which show the worst fitting (**Figs. 2** to **8**). **Table 3** shows the coefficients for the Von Bertalanffy model.
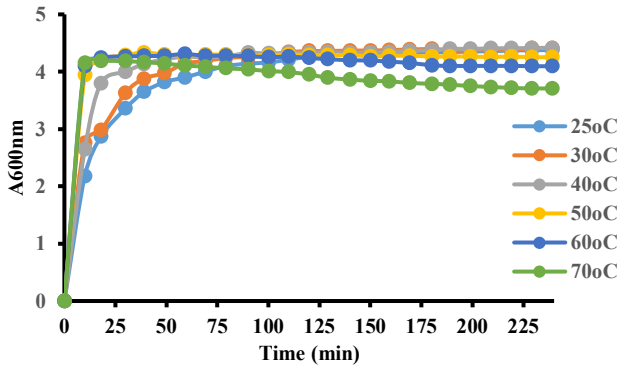
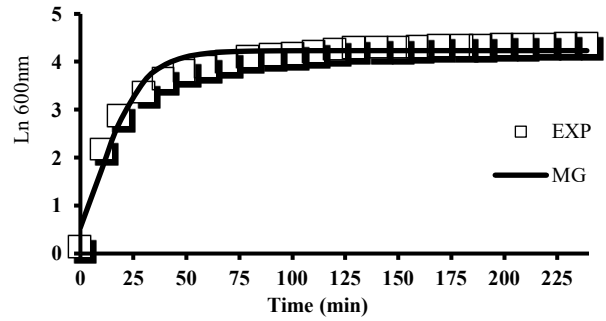**Fig. 1**. Growth of *Bacillus cereus* on malachite green at different temperatures.

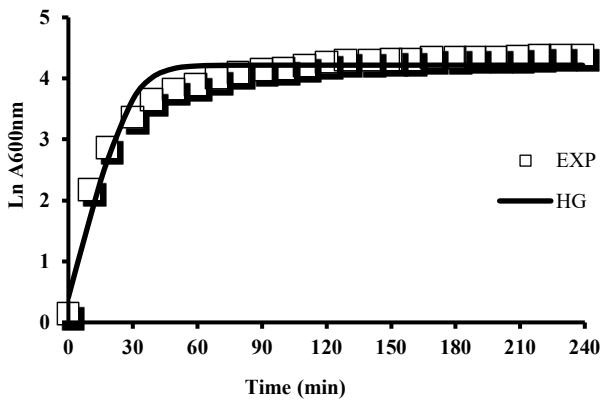**Fig 4.** Growth of *Bacillus cereus* modelled using modified-Gompertz model.

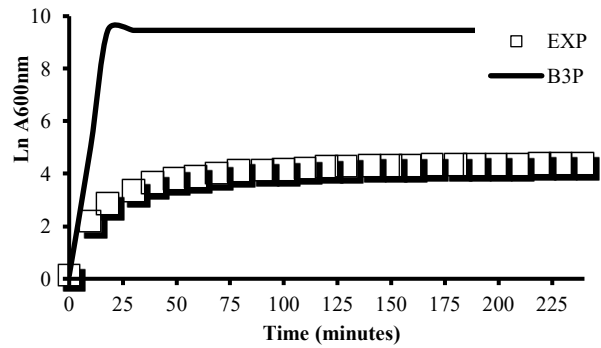**Fig. 2**. Growth of *Bacillus cereus* modelled using Huang model.

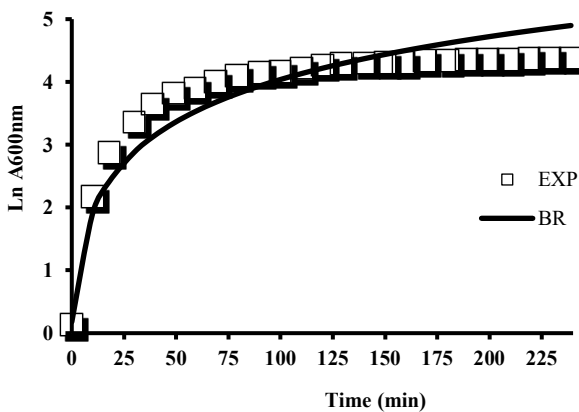**Fig. 5.** Growth of *Bacillus cereus* modelled using Buchanan-3-phase model.

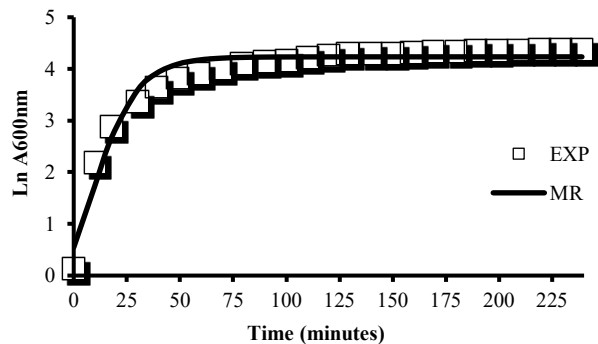**Fig. 3.** Growth of *Bacillus cereus* modelled using Baranyi-Roberts model.

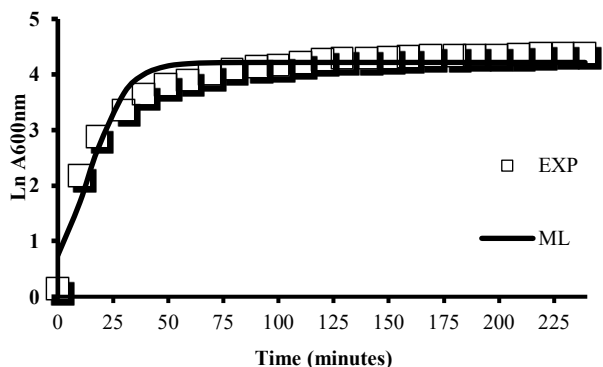**Fig. 6.** Growth of *Bacillus cereus* modelled using modified-Richards model.

Fig. 7. Growth of *Bacillus cereus* modelled using the modified-Logistics model.
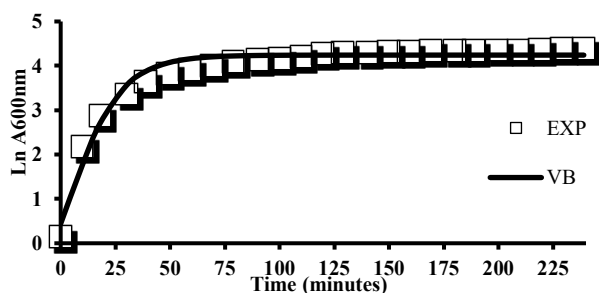

Fig. 8. Growth of *Bacillus cereus* modelled using the von Bertalanffy model.

Table 2. Statistical tests for the different models used to model the *Bacillus cereus* growth curve on malachite green dye.

Model	p	RMSE	adR2	AF	BF	AICc
Huang	4	0.2227	0.9393	1.0925	1.0349	-58.30
Baranyi-Roberts	4	0.3571	0.8892	1.0881	0.9957	-34.69
Modified Gompertz	3	0.1989	0.949	2.25	1.0482	-67.93
Buchanan-3-phase	3	5.5173	-0.045	2.25	2.2224	98.199
Modified Richards	4	0.2036	0.9465	1.0981	1.0482	-62.78
Modified Logistics	3	0.244	0.918	1.118	1.06	-57.642
Von Bertalanffy	3	0.178	0.961	1.09	1.04	-73.591

Note:
P=number of parameters

Table 3. Growth coefficients modelled using the von Bertalanffy model.

Parameters	Value	95% Confidence interval
u	0.287	0.244 to 0.329
A	2.757	2.131 to 3.382
k	4.323	4.285 to 4.362

The von Bertalanffy parameters such as *A* (lower asymptote bacterial growth), *m* (bacterial growth rate) and k (curve fitting parameter) were found to be 2.757 (95% confidence interval from 2.131 to 3.382), 0.287 (95% confidence interval from 0.244 to 0.329) and 4.323 (95% confidence interval from 4.285 to 4.361) respectively (**Table 3**). Von Bertalanffy growth model has been used to mimic the growth of a wide range of species, including fishes, mammals, birds, invertebrates, and microbes. It is a variant of Richards' model, and it is based on biological principles devised by Pütter. For many species, growth rate has been found to correlate with various life-history features, including as fertility and lifespan, making it a crucial driver of organism fitness. The model was first intended to model fish growth in ecology, but it is currently used to model other organisms, including bacterial biodegradation [43–49].

CONCLUSION

It could be concluded that, based on statistical tests such as corrected AICc (Akaike Information Criterion), bias factor (BF), adjusted coefficient of determination (R2), and root-mean-square error (RMSE), the Von Bertalanffy model was found to be the best model in modelling the growth of *Bacillus cereus* on malachite green dye. Lower asymptote bacterial growth (A), bacterial growth rate (µ), and curve fitting parameter, (k) were among the parameters obtained from the fitting exercise. The employment of bacterial growth models to achieve a precise growth rate is beneficial for secondary model development, and this work has proved the capabilities of such models.

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