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JOURNAL OF BIOCHEMISTRY, MICROBIOLOGY AND BIOTECHNOLOGY

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JOBIMB VOL 9 NO 1 2021
ARID5B GENE PRODUCT

Mathematical Modelling of the Growth of Yeast *Candida tropicalis* TL-F1 on Azo Dyes

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HISTORY

Received: 4th May 2021
Received in revised form: 12th July 2021
Accepted: 24th July 2021

KEYWORDS

Buchanan-3-phase
Azo dye
modelling
kinetics
Candida tropicalis TL-F1

ABSTRACT

Azo dyes are the most common chemical family of dyes, with a wide range of structural and color variations. They account for up to 70% of yearly dye production. Azo dyes are one of the first man-made compounds, and they are still commonly used in the food and textile industries. The discharge of Azo dyes is undesirable not only because many Azo dyes and their breakdown products are toxic to aquatic life and mutagenic to people, but also because many Azo dyes and their breakdown products are harmful to aquatic life and mutagenic to humans. We report different primary kinetics models such as Huang, modified Gompertz, Buchanan-3-phase, modified Logistics, Baranyi-Roberts, modified Richards and Von Bertalanffy were used to get the best model for *Candida tropicalis* TL-F1 growth on different Azo dyes concentrations. The best model was found to be Buchanan-3-phase with the lowest values for AICc, RMSE and the highest value for adjusted R^2. The AF and BF values were also excellent for the model with their values were the closest to 1.0. The poorest performance was found to be Baranyi-Roberts where it failed to model the growth curve. Baranyi-Roberts has the highest values for AICc, RMSE and the highest value for adjusted R^2. The Buchanan-3-phase parameters such as Y_0, λ, k and Y_{max} were found to be 0.290, 3.244, 0.543 and 3.825 respectively. These constant would provide insight for the actual *Candida tropicalis* TL-F1 growth curve.

INTRODUCTION

The most prevalent form of synthetic dye used in the textile industry is Azo dyes. These dyes are known to be xenobiotic chemicals containing electron-withdrawing groups, which cause electron deficit and therefore make them resistant to degradation [1]. Azo dyes are the most common chemical family of dyes, with a wide range of structural and colour variations. They account for up to 70% of yearly dye production [2]. The global yearly production is estimated to be around 700,000 tons [3]. Approximately, 10 - 15% of these dyes are discharged into the environment throughout the production and use process [4]. Several dyes, as well as some of their N-substituted aromatic biotransformation intermediates are carcinogenic, making them major environmental pollutants. Thus, different biological, chemical and physical techniques for not just color removal but also full mineralization of Azo dyes have been developed [5]. Biological techniques, on the other hand, were frequently employed in compared to physical and chemical approaches since that it is environmental-friendly, easily to use and cost-effective [6]. Biodegradation of microbial Azo dyes usually occurs in two phases. The dyes' Azo links are reductively cleaved in the first stage, resulting in the production of colorless but potentially dangerous aromatic amines. The aromatic amines are degraded in the second step [7]. Azo dyes reduction generally necessitates anaerobic conditions, whereas aromatic amine biodegradation is nearly entirely an aerobic process. Nevertheless, some microorganisms had been isolated that could degrade Azo dyes aerobically via Azoreductases [8]. According to Sarayu and Sandhya [9], the breakdown products could be further degraded during aerobic decolorization processes through the catalysis of monooxygenase and dioxygenase, which could induce the incorporation of oxygen atoms from O_2 into the aromatic ring of organic compounds prior to ring fission.

Studies on microorganisms that might decolorize Azo dyes were largely concentrated on bacteria, algae and fungi [10]. Among them, algae were prevalent in aquatic habitats, however the studies on their application for Azo dyes decolorization

were limited presumably because the development of algae was controlled by many particular variables such as light intensity and concentration of CO_2. Bacteria were commonly employed to decolorize Azo dyes due to their high activity, broad dispersion, and flexibility. However, decolorization intermediates such as aromatic amines may limit the action of bacteria on a wide scale. Fungi, on the other hand, produced extracellular ligninolytic enzymes that were capable of decomposing complex organic molecules [11].

Microbial growth curves usually followed a sigmoidal pattern, beginning with the lag portion soon after t = 0, then the logarithmic section, then the stationary phase, and ultimately the death phase or reduction in microbial growth. Sigmoidal functions such as modified Logistics, Modified Gompertz, Von Bertalanffy, Baranyi-Roberts, Buchanan 3-phase and Huang among others are used to characterize the yeast growth curve [12]. All sigmoidal functions were adjusted to include all biologically relevant factors. Stannard's, Schnute's, and Richards' models appeared to be essentially the same equation. The maximum specific growth rate (μ_{max}), the lag time, and the asymptotic values are all important growth curve characteristics.

The value of the maximum specific growth rate (μ_{max}) can be used to create secondary models to investigate the effects of substrate on growth rate. Because of the advantages of nonlinear regression analysis of Azo dyes degradation have been described above, the goal of this study is to compare and contrast a different primary models, including Huang model [13], Buchanan three-phase [14], Logistic [15, 16], Gompertz [16, 17], Richards [16, 18], Baranyi-Roberts [19], Von Bertalanffy [20, 21]. In this current study, we show for the first time the use of the Buchanan-3-phase model in modelling yeast growth on Azo.

MATERIALS AND METHODS

Data from Fig 2B. from Tan et al. [11] which shows the effect of initial concentration of Acid Brilliant Scarlet on growth of strain TL-F1 was processed using the software CurveExpert Professional software (Version 2.6.5) which digitizes the scanned figures into table of data with good enough precision and has been used recently.

Statistical analysis

Statistical significant difference between the models was calculated through various methods including the adjusted coefficient of determination (R^2), accuracy factor (AF), bias factor (BF), Root-Mean-Square Error (RMSE) and corrected AICc (Akaike Information Criterion) as before [22]. BF and AF were initially proposed by Ross and McMeekin [23] and are used to measure the confidence level in the prediction of a model.

It also tests a model goodness-of-fit. Bias factor that is equal to 1 signifies a best correlation between experimental and predicted values. A bias factor with value > 1 signifies a fail-safe model, whereas a BF with values < 1 signifies a fail-dangerous model. Usually, the value of AF is always greater than or equal to (≥) 1, with higher AF value signifies a less precise prediction. Bias and accuracy factor is calculated in Equations below.

Fitting of the data

Fitting of the yeast growth curve using various growth models (Table 1) was carried out using the CurveExpert Professional software (Version 2.6.5) by nonlinear regression utilizing the Marquardt algorithm. This minimizes the sums of the square of the difference between the experimental and predicted values. The software can be manually or automatically encoded to estimate parameters initial values. μmax estimation was made using the sharpest gradient search of the curve among the four datum points [24].

Lastly, the last datum point is an estimation for the asymptote (A). The Huang's model needs to be solved mathematically as it is differential equation. The Runge-Kutta method was utilized through the ode45 solver in MATLAB (Version 7.10.0499, The MathWorks, Inc., Natick, MA) [25].

Table 1. Various mathematical models developed for growth kinetics involving substrate used in this study.

Model	P	Equation
Modified Logistic	3	$y = \frac{A}{\left\{1+\exp\left[\frac{4\mu_m}{A}(\lambda - t)+2\right]\right\}}$
Modified Gompertz	3	$y = A\exp\left\{-\exp\left[\frac{\mu_m e}{A}(\lambda - t)+1\right]\right\}$
Modified Richards	4	$y = A\left\{1+v\exp(1+v)\exp\left[\frac{\mu_m}{A}(1+v)\left(1+\frac{1}{v}\right)(\lambda - t)\right]\right\}$
Modified Schnute	4	$y = \left(\mu_m \frac{(1-\beta)}{\alpha}\right)\left[\frac{1-\beta\exp(\alpha\lambda+1-\beta-\alpha t)}{1-\beta}\right]^{\frac{1}{\beta}}$
Baranyi-Roberts	4	$y = A + \mu_m t + \frac{1}{\mu_m}\ln\left(e^{-\mu_m t} + e^{-h_0} - e^{-}\right.$ $\left. - \ln\left(1 + \frac{e^{\mu_m t + \frac{1}{\mu_m}\ln\left(e^{-\mu_m t}+e^{-h_0}-e^{-\mu_m t-h_0}\right)} - 1}{e^{(y_{max}-A)}}\right)\right.$
Von Bertalanffy	3	$y = K\left[1-\left[1-\left(\frac{A}{K}\right)^3\right]\exp^{-\left(\mu_m t/3K^{\frac{1}{3}}\right)}\right]^3$
Huang	4	$y = A + y_{max} - \ln\left(e^{A} + \left(e^{Y_{max}} - e^{A}\right)e\right.$ $B(t) = t + \frac{1}{\alpha}\ln\frac{1+e^{-\alpha(t-\lambda)}}{1+e^{\alpha\lambda}}$
Buchanan Three-phase linear model	3	Y = A, if t < lag Y=A + k(t–λ), if λ ≤ t ≥ t_{max} Y = y_{max}, if t ≥ t_{max}

Note:
A= growth lower asymptote;
Y_{max}= growth upper asymptote;
μ_{max}= maximum specific growth rate;
v= affects near which asymptote maximum growth occurs.
l=lag time
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/μ_{max}

RESULTS AND DISCUSSION

Based on the bacterial growth modelling (Figures 1-7), the best performance was found to be Buchanan-3-phase with the lowest values for AICc, RMSE and the highest value for adjusted R^2. The AF and BF values were also excellent for the model with their values were the closest to 1.0. The poorest performance was Baranyi-Roberts where it failed to model the growth curve (Table 2). Baranyi-Roberts has the highest values for AICc, RMSE and the highest value for adjusted R^2. The coefficients for the Buchanan-3-phase model are shown in Table 3.

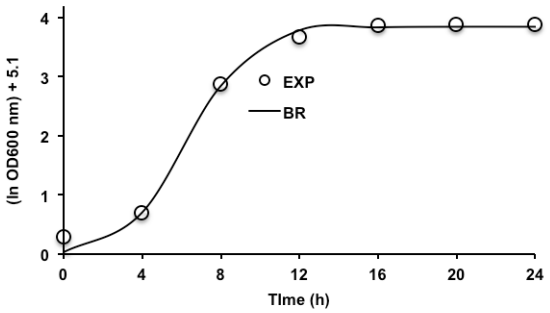

Fig. 1. Growth of *Candida tropicalis* TL-F1 as modelled using the Baranyi-Roberts Buchanan-3-phase model.

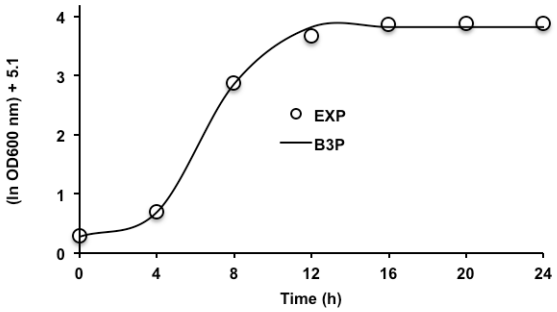

Fig. 2. Growth of *Candida tropicalis* TL-F1 as modelled using the Buchanan-3-phase model.

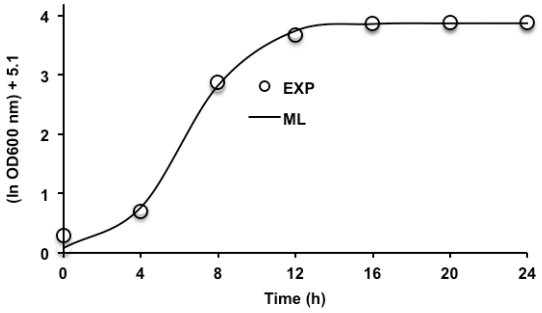

Fig. 3. Growth of *Candida tropicalis* TL-F1 as modelled using the modified Logistics model.

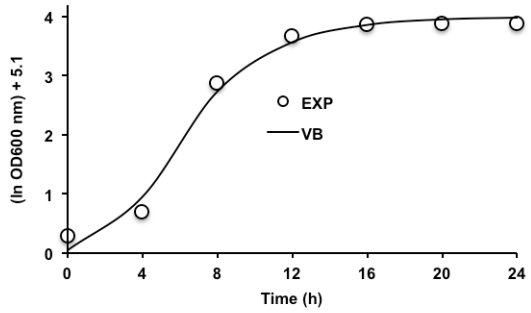

Fig. 4. Growth of *Candida tropicalis* TL-F1 as modelled using the von Bertalanffy model.

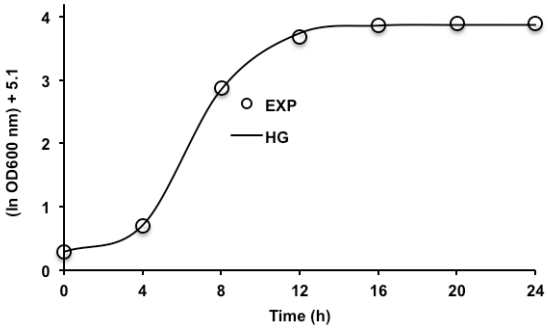

Fig. 5. Growth of *Candida tropicalis* TL-F1 as modelled using the Huang model.

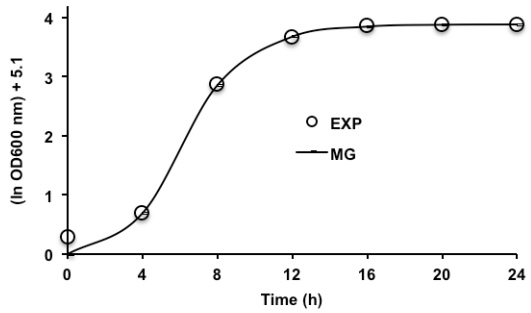

Fig. 6. Growth of *Candida tropicalis* TL-F1 as modelled using the modified Gompertz model.

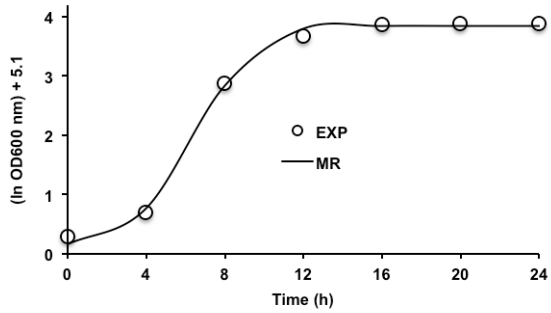

Fig. 7. Growth of *Candida tropicalis* TL-F1 as modelled using the modified Richards model.

Table 2. Statistical tests for the different primary models utilized in modelling the growth curve of *Candida tropicalis* TL-F1.

Model	*p*	RMSE	R^2	$AdjR^2$	AF	BF	AICc
Huang	4	0.04	1.00	1.00	1.01	1.00	28.47
Baranyi-Roberts	4	0.17	0.99	0.98	1.41	0.72	47.29
modified Gompertz	3	0.15	0.99	0.99	1.01	0.37	3.08
Buchanan-3-phase	3	0.09	1.00	1.00	1.01	1.00	-3.48
modified Richards	4	0.12	1.00	0.99	1.12	0.93	42.71
modified Logistics	3	0.12	1.00	0.99	1.22	0.85	0.07
von Bertalanffy	4	0.20	0.99	0.98	1.40	0.79	7.85

Note: p is no of parameter

Table 3. Growth coefficients as modelled using the Buchanan-3-phase model.

Parameter	Value	(95% confidence interval)
A or Y_0 (Log CFU/mL)	0.290	-0.041 to 0.621
κ	0.543	0.425 to 0.660
lag (h)	3.244	2.289 to 4.200
Y_{max} (Log CFU/mL)	3.825	3.659 to 3.991

The Buchanan-3-phase model gave the best fitting based on statistical test with the lowest values for RMSE the highest value for adjusted R^2 and the closest values to unity for both Accuracy and Bias factors. However, it has a corrected Akaike Information Criteria value of -3.48, which is the lowest among all other models Baranyi-Roberts model gave the poorest performance as it failed to unite and have the highest values for AICc and RMSE of 47.29 and 0.17 respectively.

Recently, Buchanan-3-phase model was introduced [14], but has found applications in modelling bacterial growth in various substrates concentrations such as the growth of *Escherichia coli*, *Listeria monocytogenes* and *Bacillus cereau* [26]. Buchanan-3-phase gave the lowest RMSE and SSE values, which are in compliance with this present study.

Parameters obtained from the curve fitting exercise were minimal growth (Y_o), lag time (λ), maximal growth (Y_{max}) and maximum growth rate (μ_{max}). These suitable constants are useful for modelling secondary kinetics most especially maximum growth rate which will give us insight such as the substrate effect on growth rate [12, 27]. It shows that, Azo dye is toxic to yeast growth based on the modelling resulting in a decrease in the maximum growth achieved as the Azo dye concentration was increase. The lag phase and maximal growth were found to be 3.244 and 3.825, respectively. These periods were not severely affected implying that possibly the cells were able to withstand the toxic effect of Azo dye at the initial growth of the yeast. Currently, primary mathematical models are mostly used as a means for quantitative food environmental and biotechnology. These models are being used in combination with curve-fitting software to analyze environmental bacterial growth. Buchanan-3-phase model is one of the commonly used model. Though, it does not take account into the underlying physiological actions and is not mechanically based.

CONCLUSION

The Buchanan-3-phase model was the best model in modelling the *Candida tropicalis* TL-F1 growth curve on Azo dye based on statistical tests such as corrected AICc (Akaike Information Criterion), bias factor (BF), adjusted coefficient of determination (R^2) and root-mean-square error (RMSE). The poorest performance was Baranyi-Roberts where it failed to model the growth curve. Baranyi-Roberts has the highest values for AICc, RMSE and the highest value for adjusted R^2. Parameters obtained from the fitting exercise were lag time (□), maximal growth (Y_{max}) and minimal growth (Y_o). These constant would provide insight for the actual *Candida tropicalis* TL-F1 growth curve.

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